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SYNTHESIS AND CHARACTERIZATION OF ELECTROSPUN GELATIN/DENDRIMER SCAFFOLD ENCAPSULATED WITH A SILVER AS A POTENTIAL ANTIMICROBIAL WOUND DRESSING

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A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering at Virginia Commonwealth University

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A novel nanofiber scaffold was fabricated and characterized as a potential antimicrobial wound dressing. Half generation polyamidoamine (PAMAM) dendrimer G3.5 was covalently conjugated to gelatin. Gelatin alone or with gelatin-dendrimer conjugates was electrospun into nanofiber scaffolds. Gelatin is a derivative of natural collagen, and it is biocompatible, non-toxic and inexpensive, making it a desirable component in a wound dressing. Dendrimers are synthetic polymers comprising of a central core, internal branches and reactive surface groups. They

provide a structurally controlled architecture for drug release. Silver was incorporated into the scaffold in situ due to its broad spectrum of antimicrobial properties. The scaffolds were further crosslinked by photo curable PEG-diacrylate in solution or vapor to gain structure stability. The fabricated scaffolds with various compositions displayed a wide range of structure characteristics and properties in terms of fiber morphology, swelling and degradation, mechanical properties, antimicrobial activity and silver release kinetics. The scaffolds showed a similar fiber structure and morphology. It was found that the fiber diameter of the scaffolds containing silver was greater than scaffolds without silver. The porosity of the crosslinked scaffolds ranged from 67.56% to 90.42% and also exhibited a high capacity of swelling and adsorption. The results of the antimicrobial assay showed that scaffolds containing silver could effectively inhibit growth of bacteria at the end of 48 h. In vitro silver release studies demonstrated that silver could be released in a controlled manner over an extended period of time.

CHAPTER 1 INTRODUCTION

Skin is the outermost layer of the body and therefore most easily susceptible to injuries or wounds. Wounds may result from a variety of sources like accidents, surgical procedures, burns, trauma, ulcers or diseases like diabetes. They need to be treated properly to ensure effective and rapid healing. Chronic wounds affect approximately 1.5 to 3 million Americans every year and account for $7 billion in healthcare worldwide (Margolis et al. 2002). Also, as the number of people suffering from diabetes is increasing, diabetic foot ulcers occur in almost 25% of the patients with diabetes (Centegra).

A number of new materials that hold great promise towards wound treatment have been developed. Biocompatible materials like hydrogels, hydrocolloids, foams, scaffolds and gauze are being actively studied for developing effective wound dressing products. Recently, electrospun nanofiber scaffolds have become a subject of attention for development of composite wound dressings. Such electrospun scaffolds can be generated from natural and synthetic biopolymers. They mimic the extracellular matrix, are biocompatible, biodegradable with highly porous structures providing space for cell in growth and air and moisture exchange. Also, therapeutics such as drugs and growth factors can be incorporated into the scaffolds for enhanced treatment.

The objective of this thesis was to synthesize and design a gelatin-dendrimer nanofiber scaffold with silver encapsulated as a potential antimicrobial wound dressing. Gelatin is a substance native to the human body. It is non-toxic, inexpensive and bio-compatible. Dendrimers are highly branched molecules comprising of a central core, internal branches and reactive surface groups. They provide a structurally stable architecture for drug delivery and controlled drug release. Silver is known for its antimicrobial properties and its use has been revived due to the emergence of antibiotic resistant organisms.

The experimental protocol involved the preparation of gelatin-dendrimer conjugates and preparation of solutions for electrospinning scaffolds. Eight different solutions were prepared for electrospinning consisting of gelatin and gelatin-dendrimer with different concentrations of silver, 0.825 mg/ml, 1.65 mg/ml and 3.3 mg/ml. To improve their structural stability, the scaffolds were crosslinked by solution or vapor of PEG-diacrylate in the presence of

dimethoxyphenylacetophenone (photo-initiator) upon irradiation. The morphology, structure and fiber diameter of the scaffolds were examined by scanning electron microscopy (SEM). The crosslinked scaffolds were further characterized by determining the mechanical strength, porosity, permeability and swelling ratio. The antimicrobial activity of the scaffolds was investigated against two common wound pathogens: gram-positive *Staphylococcus aureus* and gram-negative *Pseudomonas aeruginosa.* Data were collected at 4 h, 24 h and 48 h time points. It was observed that the scaffolds with all concentrations of silver could inhibit the growth of bacteria efficiently at the end of 48 h. The in-vitro release kinetics of silver was studied by Inductively coupled plasma-optical emission spectroscopy (ICP-OES).

CHAPTER 2 BACKGROUND

2.1 Phases of wound healing

A wound in the skin can be generated by physical or thermal damages or as a result of a medical condition (Boateng et al. 2008). Wounds may be classified as acute or chronic wounds. Acute wounds are injuries that heal within 8 to 12 weeks with minimum scar formation, whereas chronic wounds do not heal within 12 weeks (Boateng et al. 2008; Harding et al. 2002; Nicholas 2002).

Normal wound healing is a natural, biological process that leads to the growth and regeneration of tissue (Boateng et al. 2008). It involves platelets, neutrophils, macrophages, lymphocytes, fibroblasts, epithelial cells, growth factors and etc (Epstein et al. 1999). The normal process of wound healing consists of four phases namely: (i) Hemostasis, (ii) Inflammatory phase, (iii) Proliferative phase, and (iv) Maturation or remodeling phase (Ratner 2004). A schematic diagram of the process is shown in Figure 1 and the phases are described as:

2.1.1 Hemostasis

The immediate reaction of our body, to a cut or injury to the skin, is bleeding from the injured blood vessels (Boateng et al. 2008). Bleeding activates the platelets and clotting factors that play an important role in hemostasis. The clotting cascade is activated and fibrinogen and thrombin form a stable fibrin clot to prevent further bleeding. Further, the clot protects the underlying injured tissue (Martin 1997; Ratner 2004). Platelets secrete certain cytokines and growth factors such as platelet derived growth factor (PDGF) and transforming growth factor- β (TGF- β) to help in the healing process (Epstein et al. 1999).

2.1.2 Inflammatory phase

The inflammatory phase is characterized by swelling and redness at the area of injury. Neutrophils, lymphocytes and macrophages play an important role in inflammation by phagocytizing the bacteria and micro-organisms (Keast and Orsted). The release of plasma proteins at the wound site also causes vasodilation of the blood vessels (Boateng et al. 2008). Macrophages produce chemotactic and growth factors like interleukin-1 (IL-1), platelet derived

growth factor, transforming growth factor- β (TGF- β), epidermal growth factor, fibroblast growth factor and insulin-like growth factor which promote the proliferative phase of wound healing (Faler et al. 2006; Keast and Orsted).

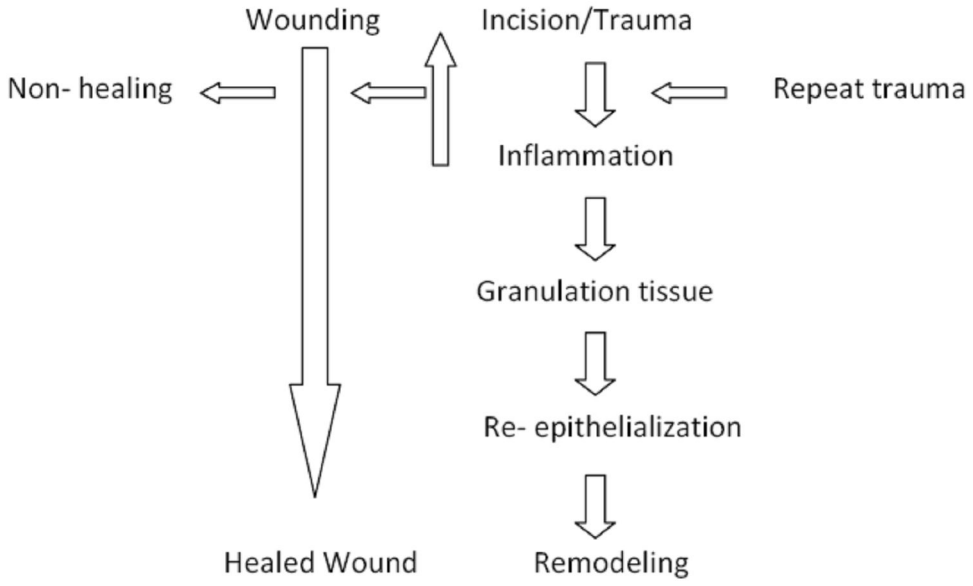
2.1.3 Proliferative phase

The proliferative phase begins when fibroblasts enter the wound site and involves extracellular matrix (ECM) deposition, angiogenesis and epithelialization (Faler et al. 2006). Fibroblasts secrete collagen which is helpful for cell migration and proliferation (Faler et al. 2006). Epithelialization occurs with formation of new blood vessels.

2.1.4 Maturation or remodeling phase

This is the last phase of the healing process and may take from about several months to years to complete depending on wound type (Boateng et al. 2008). Fibroblasts facilitate the formation of a collagen network and remodeling to strengthen the scar.

Figure 1: Phases of wound healing (Re-drawn from (Percival and Keith 2010))



2.2 Factors affecting wound healing

There are various factors that can result in an impaired wound healing (Guo and DiPietro 2010) and generally lead to a pathological wound (Boateng et al. 2008). Such factors can be classified as local and systemic factors (Guo and DiPietro 2010). Local factors are those that directly affect the wound (Guo and DiPietro 2010) such as infection, ischemia, oxygenation, topical agents, or foreign bodies (Guo and DiPietro 2010; Kerstein 1997; Waldorf and Fewkes 1995), whereas systemic factors are related to the health state of an individual, such as age, nutrition, diseases (diabetes, fibrosis, jaundice etc.), medications, smoking, or immunocompromised disorders (cancer, radiotherapy) (Guo and DiPietro 2010). Bacteria like *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyrogenes* and *Proteus, Clostridium and Coliform* species are common wound pathogens and unfavourable (Boateng et al. 2008; Guo and DiPietro 2010). Studies have shown that skin graft healing was affected by *P. aeruginosa* and *S. aureus* and the presence of *S. aureus* was found in 94% of the ulcers (Gilliland et al. 1988). Production of excess collagen results in the formation of keloid scars (Martin 1997). Age also affects the healing process since aging decreases the ability to combat infection (Boateng et al. 2008). Also, poor nutrition such as vitamin, mineral and protein deficiency impair and delay the wound healing process (Hemila and Douglas 1999; Patel 2005; Rojas and Phillips 1999). Diseases like diabetes affect wound healing due to poor blood circulation and also they may delay inflammation and production of collagen (Boateng et al. 2008).

2.3 Drugs, growth factors and other therapeutics to enhance the efficacy of the dressing

Apart from developing dressings to protect wounds, research is being conducted to incorporate therapeutics into wound dressings. These incorporated agents play an active part in the wound repair process. For example, antimicrobial agents can be included to inhibit growth of micro-organisms (Boateng et al. 2008).

As antimicrobial agents are able to inhibit growth of micro-organisms and prevent infection in the wound area, they are mainly for diabetic foot ulcers (Nelson et al. 2006; O'Meara et al. 2000), or wounds resulting from surgery or accidents (Harihara et al. 2006), where the risk of infection is high (Boateng et al. 2008). Commonly used antimicrobial agents are povidone-iodine with fabric dressings, silver with synthetic dressings, gentamycin with collagen dressings and

ofloxacin with silicone gel sheets (Boateng et al. 2008). The delivery of antibiotics to the wound site through wound dressings is preferred over systemic administration since the dosage required for the latter may lead to toxic reactions in the body or may be ineffective due to poor blood circulation to the extremities (Boateng et al. 2008) (e.g., diabetic foot ulcers). In addition, delivery of antibiotics through wound dressings decreases the occurrence of bacterial resistance and interference with the wound repair process (Doillon and Silver 1986).

Growth factors play an active role in cell division, migration, differentiation and proliferation (Boateng et al. 2008). They can be incorporated into wound dressing to enhance the healing process by (i) increasing the activity of inflammatory cells and fibroblasts at the wound site and (ii) stimulating cellular proliferation and angiogenesis, which, in turn, affects the production and degradation of the extracellular matrix (Greenhalgh 1996; Komarcevic 2000). Various growth factors that have been identified to play a role in wound healing are epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor-β (TGF- β) and insulin like growth factor (IGF-1) (Greenhalgh 1996; Steenfos 1994). Different dressings have been investigated for delivering specific growth factors. Collagen dressings have been shown to effectively deliver EGF (Grzybowski et al. 1999) and PDGF (Koempel et al. 1998) and hydrogel dressings have been shown to deliver TGF-β (Puolakkainen et al. 1995). Research done by Park et al. (Park et al. 2004) showed that wound healing was more effective when a collagen dressing containing antibiotics and FGF was used as compared to collagen dressing with antibiotic only.

2.4 Wound dressings

An ideal wound dressing is one that promotes wound healing and provides optimal environment for wound healing to take place (Shanmugasundaram et al. 2006). The desired characteristics of a wound dressing are (Ngan 2005):

- Maintaining optimum temperature and environment for wound healing
- Protecting the wound from any injury and infection by bacteria or other micro-organisms
- Controlling the moisture content
- Providing mechanical support to the wound site

- Absorbing wound exudates and keeping wound area clean
- Sterility and non-toxicity

Wound dressings can be classified based on the type of material, function and physical form of the dressing (Boateng et al. 2008):

Table 1: Classification of wound dressings (Boateng et al. 2008; Ngan 2005; Paul and Sharma 2004)

Type of Dressing	Characteristics	Example(s)
Passive Products	Comprise of traditional dressings	Gauze and tulle dressings
Interactive Products	These are dressings composed from polymers which allow moisture and oxygen exchange but can keep bacteria out. These are mostly applied to wounds that produce low exudates	Hydrogels and foam dressings
Bioactive Products	These comprise of dressings which deliver substances that can enhance the wound healing process	Alginates, chitosan, collagens and hydrocolloids

2.4.1 Traditional and modern dressings

Wound dressings have evolved over the period of time from raw applications like medicinal plants/herbs, honey and etc., to commercially designed dressings (Boateng et al. 2008). Although medicinal herbs and plants have been shown to demonstrate antimicrobial activity and reduce inflammation, their direct application may be harmful as they could contain micro-organisms which would be a source of infection (Boateng et al. 2008). Therefore, it is important to maintain an aseptic environment for wound healing to take place and use sterile wound dressings (Boateng et al. 2008). The research done by Winter in the 1960s led to the development of an approach to maintaining a moist environment for enhanced wound repair (Winter 1962). This boosted the development of advanced dressings with added functionalities to enhance wound healing (Sai and Babu 2000).

Traditional dressings consist of gauze, bandages or materials woven from fabric (Boateng et al. 2008; Sai and Babu 2000). They are dry dressings and hence moisture content can easily

evaporate through them, thus not providing a moist environment for healing. They cannot inhibit entry of micro-organisms and also its adherence to the wound causes difficulty in removing the dressing. To overcome these drawbacks, tulle dressings, gauze meshes impregnated with paraffin, have been developed. They can be removed from skin with less pain (Boateng et al. 2008; Sai and Babu 2000). However, traditional dressings need to be changed frequently and are less cost effective than synthetic/modern dressings (Harding et al. 2000). Due to these disadvantages, modern or synthetic dressings are being actively researched and developed for wound treatment.

Synthetic or modern wound dressings are classified based on the starting material used. They include hydrocolloids, alginate, hydrogel, foam, biological dressings (Boateng et al. 2008). Hydrocolloid dressings are formed from colloidal (gel-like) substances added to adhesive compounds. They can be obtained in the form of thin films or sheets and are useful for treating light to moderately exuding wounds (Boateng et al. 2008). In the original state, hydrocolloid dressings are impermeable to water vapor. However, in the presence of wound exudates, the dressings change their structure to a gel-like covering hence becoming more permeable to water and oxygen (Thomas and Loveless 1997). They do not cause any pain upon removal and are particularly useful in pediatric wound care (Thomas 1992). Alginate dressings are composed of calcium alginate and are useful in moderate to heavily exuding wounds like bleeding (Boateng et al. 2008; Paul and Sharma 2004). They can be used in the form of fibers or foams (Boateng et al. 2008). Similar to hydrocolloids, alginate dressings also can form a gel upon contact with the wound exudates. Upon application, ion exchange takes place between the exudates and alginate fibers to help form a protective gel (Thomas 2000). This helps control the moisture content and minimizes the pain during removal of the dressing (1994; Boateng et al. 2008; Gilchrist and Martin 1983).

Hydrogels are hydrophilic substances composed of synthetic polymers and have high swelling capacity (Boateng et al. 2008; Sai and Babu 2000). They occur in the form of amorphous gel or as an elastic sheet. Hydrogel in the form of elastic sheet contains crosslinked polymers so that it can retain water (Boateng et al. 2008; Sai and Babu 2000). They do not react with biological tissues, are permeable to water and metabolites (Wichterle and Lim 1960), provide a moist and

cool environment at the surface of the wound to reduce pain (Boateng et al. 2008; Sai and Babu 2000) and promote reepithelization of wounds (Debra and Cheri 1998). Foam dressings are sheets composed of polymer solutions like polyurethane (Chardack et al. 1962), which provide a moist environment and thermal insulation at the wound site (Boateng et al. 2008). These dressings are highly porous in nature, can absorb large amounts of fluid and hence can be used for low, moderate or heavily exuding wounds (Thomas 1990). Biological dressings comprise dressings derived from natural tissues or tissue engineered products consisting of combination of polymers such as collagen, hyaluronic acid, chitosan and elastin (Bartlett 1981). The major characteristics that make them attractive components for wound healing, are biodegradability, biocompatibility, non-toxicity and an active role in promoting new tissue formation (Boateng et al. 2008; Kollenberg 1998; Ueno et al. 1999).

2.4.2 Drug delivery for wound healing

Drug delivery systems have been developed to increase the effectiveness of therapeutic drugs through controlled release. Local drug delivery can be achieved by encapsulating the drug into biopolymer matrices or by covalent attachment to dendrimer surface groups or other polymers (Saltzman and Olbricht 2002). Controlled delivery of antibiotics to the wound site is beneficial because it reduces the need to deliver high doses of antibiotics systemically (Boateng et al. 2008). Dressings made from natural or synthetic biopolymers can be incorporated with drugs and growth factors for effective healing. Many polymeric dressings have been evaluated for controlled drug delivery including hydrogels based on poly(vinyl alcohol), poly(lactide-co-glycolide), poly(vinyl pyrrolidone), poly(hydroxyl-alkylmethacrylates) and alginate, polymeric matrices prepared from hyaluronic acid, collagen or chitosan and synthetic dressings which include silicone gel sheets (Boateng et al. 2008).

Controlled drug release at wounds is governed by swelling of the polymer matrix, diffusion of the drug, degradation of the scaffold or the combination of the above (Boateng et al. 2008; Sill and von Recum 2008). Diffusion of the drug and degradation of the scaffold are desirable but also disadvantageous because it may result in accumulation of the drug in large doses which can be toxic (Sill and von Recum 2008).

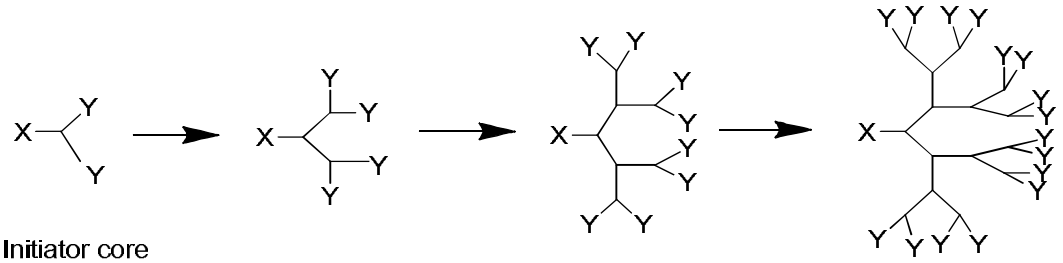
2.5 Dendrimers

Dendrimers are a group of highly branched polymers suitable for numerous applications in nanomedicine and nanotechnology. Dendrimers were first developed by Tomalia et al (Tomalia et al. 1985). The term ‘dendrimer’ is derived from the Greek word ‘dendron’ meaning tree. They are globular in structure and comprise a central core, internal branches and many reactive surface groups. There are many properties of dendrimers that make them attractive for biomedical applications: (i) they are monodisperse macromolecules (consistent size and form); (ii) they have low polydispersity index; (iii) they are highly soluble and miscible due to their branched structure; (iv) drug molecules can be encapsulated in their central core or covalently attached to their surface groups; and (v) their structurally stable architecture permits controlled drug release (Klajnert and Bryszewska 2007).

2.5.1 Synthesis of dendrimers

Dendrimers can be synthesized by two major methods: divergent synthesis and convergent synthesis (Klajnert and Bryszewska 2007). In the divergent synthesis method (Figure 2), the dendrimer advances in the outward direction from a core molecule. The core molecule reacts with monomer molecules resulting in first generation of the dendrimer. Subsequent reactions with many monomers lead to the formation of successive generations of the dendrimer. The divergent method can be used for producing large quantities of dendrimers but faces difficulty in purifying the final product.

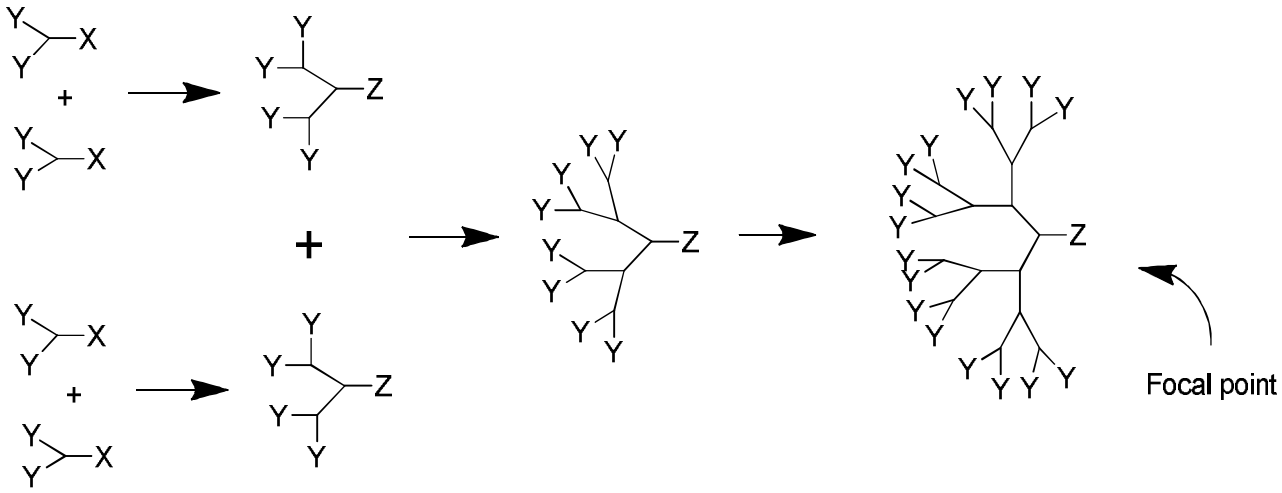
Figure 2: Divergent method for synthesis of dendrimers



In the convergent synthesis method (Figure 3), the dendrimer progresses inwards towards the core molecule. Two or more dendrons attached to the monomer molecules react with the core

molecule to form the dendrimer. The structure resulting from convergent synthesis has less defects and the final product is easier to purify. Due to steric hindrance, high generations of dendrimers are difficult to form by the convergent synthesis.

Figure 3: Convergent method for synthesis of dendrimers



2.5.2 Polyamidoamine (PAMAM) dendrimers

PAMAM dendrimers can be synthesized by divergent synthesis to have an ethylenediamine (EDA) or an ammonia core with methyl acrylate and ethylene diamine branches (Klajnert and Bryszewska 2007; Tomalia et al. 1985). The reaction scheme for the synthesis is illustrated below (Klajnert and Bryszewska 2007):

$NH_2CH_2CH_2NH_2 + 4CH_2CHCOOCH_3 \rightarrow NCH_2CH_2N\,(CH_2CH_2COOCH_3)_4$ Step 1

$NCH_2CH_2N\,(CH_2CH_2COOCH_3)_4 + 4NH_2CH_2CH_2NH_2 \rightarrow$

$NCH_2CH_2N\,(CH_2CH_2CONHCH_2CH_2NH_2)_4 + 4CH_3OH$ Step 2

Full generation (cationic) PAMAM dendrimers have terminal amine groups, whereas half generations (anionic) have terminal carboxyl groups as shown in Figure 4 and Figure 5, respectively (Klajnert and Bryszewska 2007). As the number of generations increase, the number of reactive surface groups is doubled (Klajnert and Bryszewska 2007).

Figure 4: Generation 2.0 PAMAM dendrimer

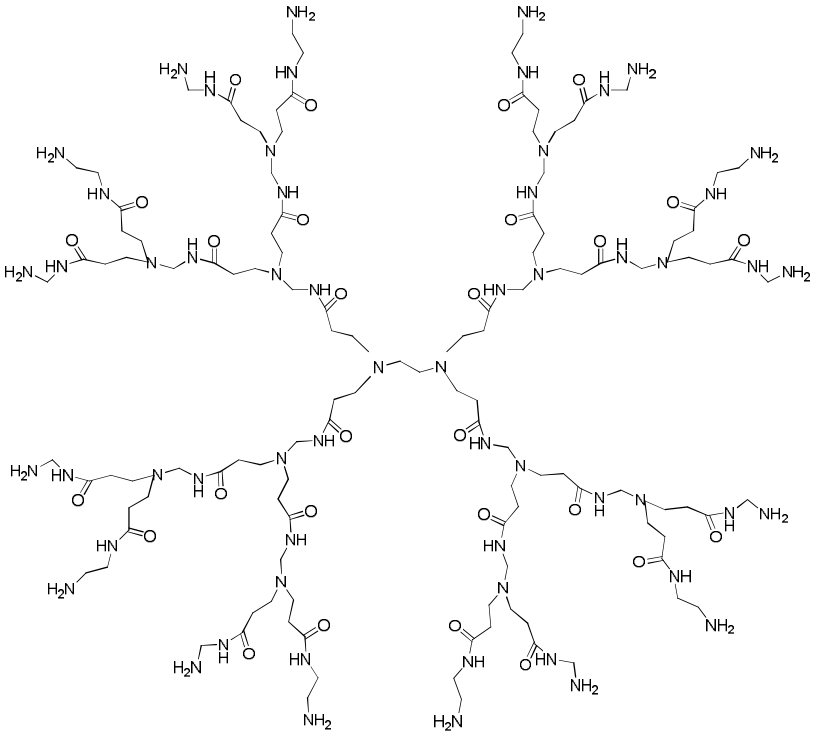
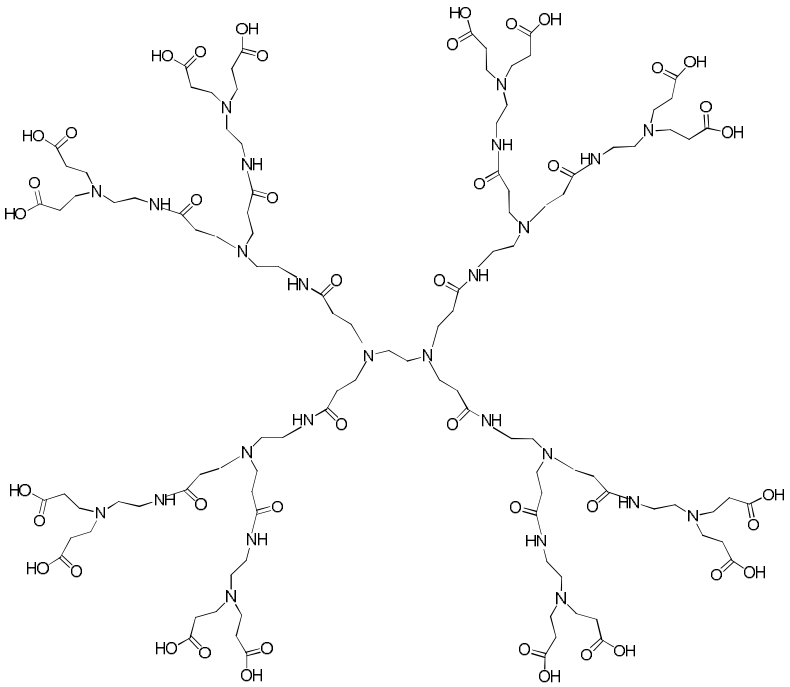


Figure 5: Generation 1.5 PAMAM dendrimer



2.6 Electrospun gelatin/dendrimer scaffold with silver

Electrospinning is a popular technique used for the fabrication of nanoscale structures for various applications like wound dressings, drug delivery vehicles and tissue engineered scaffolds (Huang et al. 2004). The scaffolds produced from natural, biodegradable polymers have very small fiber diameter ranging from nano to micrometers which is suitable to replicate the structural morphology of the natural extracellular matrix of native tissues and organs (Huang et al. 2004).

In this study, gelatin was the major component used since it is a natural biopolymer derived from collagen. It is biocompatible, biodegradable and can be commercially available at a relatively low cost (Zhang et al. 2005). It is popularly used in the field of medicine as a sealant for vascular prosthesis and as a wound dressing. However, gelatin is easily soluble in water and electrospun gelatin fibers can easily lose their structural stability in an aqueous medium. Hence, gelatin based scaffolds need to be crosslinked or incorporated with stabilizing polymers to retain its mechanical integrity as a tissue engineered construct (Zhang et al. 2005). Dendrimer can be covalently bound to gelatin and electrospun into a scaffold for drug encapsulation and drug delivery.

Silver was selected as an antimicrobial agent due to its broad range of antimicrobial activity against gram-positive and gram-negative bacteria (Hromadka et al. 2008). It can also inhibit antibiotic resistant bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE) when used at proper concentrations (Warriner and Burrell 2005). Silver can kill micro-organisms by multiple mechanisms, it can change the structure and function of a bacterial cell by altering its protein structure or rupture the bacterial cell wall or block the respiratory pathway (Warriner and Burrell 2005). Silver based wound dressings and creams are used for wound healing and to maintain a microbe free environment at the wound site (Warriner and Burrell 2005). Silver based dressings are particularly used in burn wounds, chronic leg ulcers, diabetic wounds and traumatic injuries (Ip et al. 2006). There are various ways by which silver can be incorporated in the dressing such as silver nitrate, silver sulfadiazine, silver calcium phosphate or in the form of nanocrystalline silver (Warriner and Burrell 2005). The silver can be released from the dressing by means of diffusion to the surface of the wound (Agarwal et al. 2009).

Apart from the advantages of including silver in a dressing, there are certain difficulties faced with current topical silver dressings such as slow rate of release, staining at the wound area, rapid consumption of silver ions and patient comfort (Warriner and Burrell 2005). Due to these issues, silver based dressings need to be investigated and an optimum concentration of silver introduced into the dressing needs to be selected.

CHAPTER 3 EXPERIMENTAL MATERIALS AND METHODS

3.1 Materials

Table 2: List of materials

Material	Abbreviation
Polyamidoamine (PAMAM) dendrimer generation 3.5, 10 wt% solution in methanol	G3.5
Gelatin from porcine skin- Type A	
Sodium bicarbonate	$NaHCO_3$
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide	EDC
N-Hydroxysuccinimide	NHS
Ethyl ether (anhydrous)	
Ethanol (denatured)	
Poly (ethylene glycol) diacrylate (M_n =575 g/mol)	PEG-diacrylate
Dimethoxyphenylacetophenone, 99%	DMPA
Ninhydrin, 97%	
De-ionized water	DI water
Silver acetate	
1,1,1,3,3,3 Hexafluoro-2-propanol	HFP
Sodium chloride	NaCl
Peptone	
Tryptone	
Yeast extract	
Agar	
Phosphate buffered saline	PBS
Aqueous silver standard for ICP-OES	

3.2 Equipment

Table 3: List of equipment

Equipment name	Purpose
Rotary evaporator, LABOROTA 4000 (Heidolph)	To distill low boiling point chemicals from a mixture of compounds
Flexi-dry MP controlled rate freezer (FTS Systems, Inc.)	To freeze dry the sample
Weighing scale	To measure the quantity of chemicals
Long wave ultra-violet lamp, model B (UVP)	For UV irradiation
Ultra violet visible (UV-Vis) spectrophotometer (Genesys 6, Thermo electron Corporation)	To measure the absorbance value or standard curve
Electrospinner	To electrospin scaffolds
MTS Bionix 200 - Mechanical testing system	To measure stress, strain and modulus
Scanning electron microscope model EVO 550	To take images of the scaffold and measure the fiber diameter by Image Tool
Autoclave	To sterilize medium
Incubator	To control temperature and humidity of microbial culture
Hood	To maintain sterile conditions
Eppendorf centrifuge model-5415D	To separate mixture of compounds based on density
Varian vista MPX, ICP-OES	To measure the concentration of metals in a sample by inductively coupled plasma-optical emission spectroscopy

3.3 Experimental methods

3.3.1 Preparation of gelatin-dendrimer conjugates

The protocol used for the preparation of gelatin-dendrimer conjugate was a slight modification to that used by Alicia Smith Freshwater (Smith-Freshwater 2009). Gelatin was conjugated with half generation PAMAM dendrimer G3.5. Briefly, 120 µl of G3.5 in methanol stock solution was dried by rotary evaporation and re-dissolved in 2 ml of distilled water. This solution was vortexed thoroughly and mixed with 3 mg of NHS and 5 mg of EDC while stirring for 24 h to achieve surface activated G3.5 (i.e., G3.5-NHS). To prepare the gelatin solution, 20 mg of gelatin was added to 20 ml of 0.1N $NaHCO_3$ solution and completely dissolved by stirring at 80°C until it formed a clear solution. At 24 h, the gelatin solution was added to G3.5-NHS solution and kept in an ice bath for 4 h. It was then centrifuged for 20 min at 10 rpm and the supernatant was added drop wise to 50 ml of ethyl ether and refrigerated for 24 h. It was then centrifuged for 20 min at 10 rpm and the precipitate was collected. The precipitate was further purified by rapid dialysis using 12-14 kDa MWCO dialysis tubing. The purified solution was lyophilized by FTS to obtain gelatin-dendrimer conjugates.

3.3.2 Preparation of sample solutions for electrospinning

Eight different sample solutions were prepared using HFP as a solvent. The types of scaffolds electrospun and their constituents are summarized in Table 4. The reason for electrospinning eight different scaffolds with varying amounts of silver was to compare the characteristics of these scaffolds against each other and to determine the optimum silver composition against infection. The solutions were mixed thoroughly for 24 h on a shaker plate prior to electrospinning.

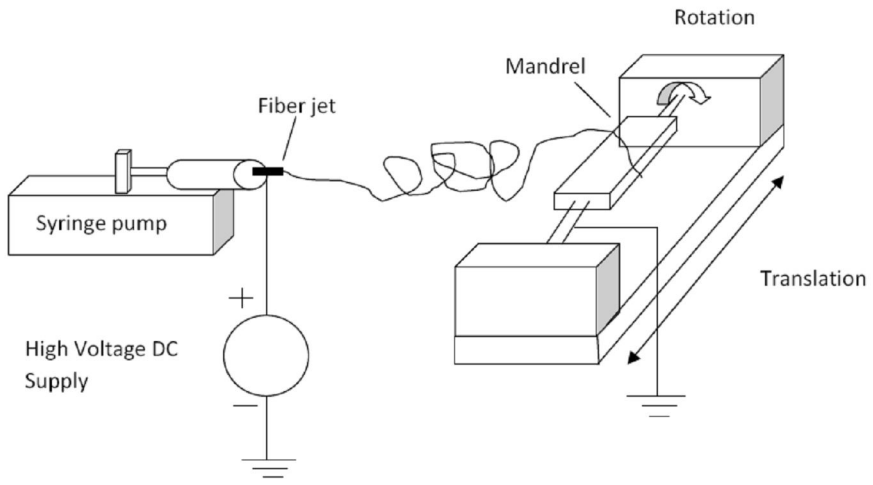
3.3.3 Electrospinning

A schematic diagram of the electrospinning technique is illustrated in Figure 6. In the electrospinning process, electrical charge is applied to draw fine fibers from the solution. The solution for electrospinning is loaded into a syringe and a positively charged electrode is attached to the needle of the syringe. The voltage applied results in an electric field and the drop of

Table 4: Solutions prepared for electrospinning scaffolds (The solutions were prepared in 10 ml of HFP)

Scaffold	Gelatin (mg/ml)	Gelatin-dendrimer conjugate (mg/ml)	Silver acetate (mg/ml)
S1	100	0	0
S2	100	0	3.3
S3	100	0	1.65
S4	100	0	0.825
S5	96	4	0
S6	96	4	3.3
S7	96	4	1.65
S8	96	4	0.825

Figure 6: Schematic diagram of electrospinning (Re-drawn from (Sill and von Recum 2008))



polymer solution at the tip of the needle is altered into a conical shape known as the Taylor cone. As the strength of the electric field increases, the polymer solution jet is elongated to form long, thin fibers as a result of solvent evaporation. The fibers are collected on a collector or mandrel that is grounded. The mandrel undergoes translation and rotation for the uniform deposition of the scaffold.

Particularly, the electrospinning solution was loaded into a 10 ml Becton Dickinson syringe and placed in a KD Scientific syringe pump for electrospinning. The syringe pump was set to deliver the solution at a rate of 5 ml/h. A voltage of 25 kV was applied to the needle of the syringe by a high voltage power supply (Spellman CZE1000R, Spellman High Voltage Electronics Corporation). The mandrel chosen for collecting the fibers was a flat, stainless steel mandrel 7.5 cm x 2.5 cm x 0.5 cm (L x W x T). It was placed approximately 125 mm from the needle tip and rotated at ~500 rpm for uniform collection of the fibers. After electrospinning was completed, the scaffold was carefully removed from the mandrel, placed in a fume hood for degassing and stored in a moisture free environment.

3.3.4 Crosslinking

After electrospinning, the scaffolds were crosslinked to increase structure stability and mechanical properties. For each scaffold, 100 µl of PEG diacrylate, 4 mg of dimethoxyphenylacetophenone (photo-initiator) and 2 ml of ethanol were used to prepare the crosslinking solution. The solution was poured onto a scaffold of 7.5 cm x 5 cm and of varied thickness and allowed to stay for about 30 min. The scaffold was then held under UV light for 2 min on each side. This method is referred to as the solution method. As an alternative method, vapors were used for crosslinking the scaffolds. The solution was heated in a water bath and the scaffold was crosslinked by the vapors. It was then held under UV light for 2 min on each side. This method is referred to as the vapor method. The scaffolds crosslinked by the vapor method did not retain their structure in aqueous medium and could be only characterized for morphology, fiber diameter, and tensile properties. Their data is shown in Appendix C.

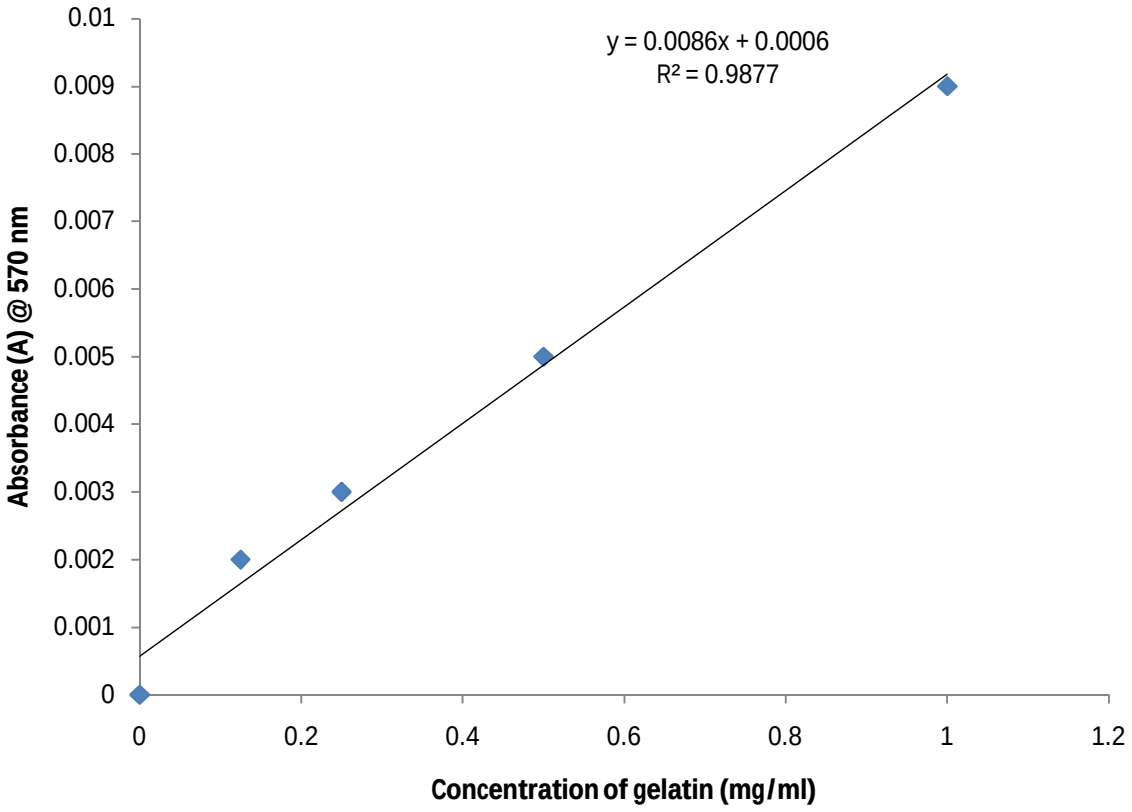
3.4 Characterization

3.4.1 Ninhydrin assay

Ninhydrin assay was performed to confirm the conjugation of dendrimer to gelatin. The ninhydrin stock solution was prepared by dissolving 30 mg of ninhydrin in 10 ml of ethanol. Five different concentrations of gelatin were prepared and mixed with 1 ml ninhydrin solution and a standard curve (Figure 7) was obtained using UV-Vis spectrophotometer. 1 mg of G3.5-gelatin conjugate was mixed with 1 ml of DI water and 1 ml of ninhydrin solution. This mixture

was heated to approximately 80°C for 5-10 min and cooled to 20-25°C and the absorbance was measured at 570 nm. The absorbance value of G3.5-gelatin conjugate mixed with ninhydrin was compared to the standard curve of gelatin mixed with ninhydrin.

Figure 7: Standard curve of gelatin (Ninhydrin assay)



3.4.2 Scanning electron microscopy (SEM)

SEM images of the scaffolds were taken to characterize the structure and morphology of the scaffolds. A small piece of sample from each scaffold was cut and gold sputter coated. Images were taken by Scanning Electron Microscope Model 550 at a magnification of 1200x. A scale bar of 10 µm is presented on each figure. Fiber diameter was calculated by using the UTHSCSA Image tool Version 3.0 to measure 30 randomly chosen fibers.

3.4.3 Tensile testing

Tensile studies of the scaffold were performed to analyze the mechanical properties of the scaffolds. Tensile studies were done on the MTS Bionix 200®- Mechanical testing system with a 100 N load cell. Six dog-bone shaped samples were cut out from each scaffold using a punch die. The thickness of the samples was measured in inches and the scaffolds were placed in the metal grips of the mechanical testing system moving at a rate of 10 mm/min. Stress, strain, modulus and energy to break were measured by the MTS Testworks software (version 4.04A).

3.4.4 Porosity measurements

Scaffold porosity was measured by taking out 1 cm x 1 cm samples from the scaffold and measuring the mass in g and thickness which ranged from 0.032 cm to 0.33 cm. Porosity was calculated by the formula:

$$P = \left[1 - \frac{V_g}{V_a}\right] \times 100$$

Where, V_g = Mass of scaffold/ Density of collagen (1.41 g/cm^3)

V_a = Apparent volume of the square section 1 cm x 1 cm x thickness

Three samples (n=3) were used from each type of scaffold for the porosity measurements.

3.4.5 Permeability and pore size measurements

Permeability was measured by an apparatus designed by Scott Sell (Sell et al. 2008). 12 mm discs were punched out from the scaffolds and the time taken for 10 ml of water to pass through the disc was noted.

Permeability was calculated as (Carr and Hardin 1987):

$$\tau = \frac{Q\mu T}{tAP}$$

Where, τ = Permeability measured in Darcy

Q=volume flowing through the system

T= scaffold thickness

μ= fluid viscosity (0.89 cP)

t = time taken for 10 ml water to flow through the scaffold (in seconds)

A= cross sectional area of scaffold (πr^2)

P= applied pressure (ρgh) in atm

Where, ρ = density of water (1000 kg/m^3)

g = gravitational force (9.8 m/s^2)

h = total height of the system (m)

The average pore size was calculated as (Carr and Hardin 1987):

$$r = \frac{0.5093}{\tau^{-1/2}}$$

Three samples (n=3) were used from each type of scaffold for permeability measurements.

3.4.6 Adsorption and swelling studies

Simulated wound fluid (Parsons et al. 2005) (SWF) was used as the medium for swelling studies and antimicrobial activity to mimic the clinical conditions. SWF consists of 50% calf serum and 50% maximum recovery diluent (0.1% w/v peptone and 0.9% w/v sodium chloride) (Parsons et al. 2005). 2.5cm x 2.5 cm of samples were cut out from each of the scaffolds and weighed (W_d). They were immersed in 5 ml of SWF at room temperature. The samples were taken out of the fluid, blot dried and weighed (W_s) at 10 min, 20 min, 30 min, 60 min, 90 min, 120 min, 24 h and 48 h. The swelling ratio (%) was calculated by the formula (Parsons et al. 2005):

$$\text{Swelling ratio \%} = \left[\frac{W_s - W_d}{W_d}\right] \times 100$$

Three samples (n=3) were used from each type of scaffold for swelling studies.

3.4.7 In vitro degradation studies

The *in vitro* degradability of the scaffolds without silver (S1 and S5) was evaluated. Four different media and conditions were used for the degradation studies: (i) incubation in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C, (ii) DMEM supplemented with 10% FBS at room temperature, (iii) incubation in SWF at 37°C and (iv) incubation in cell conditioned medium at 37°C (Cell conditioned medium was DMEM supplemented with 10% FBS used for culturing confluent BJ-hTERT fibroblasts for 24 h in 96 x 16 mm sterile petri dish) . Nine samples from each scaffold of size 1 cm x 1 cm were weighed and immersed in 1.5 ml of each of the media mentioned above, for 24 h. After 6 h, 12 h and 24 h three samples of each scaffold type were taken out from all media, centrifuged for 20 min, frozen, then lyophilized and weighed. The average ratio of weight loss due to the degradation in each scaffold was calculated using the formula:

$$\text{Ratio of weight loss (\%)} = \left[\frac{W_o - W_d}{W_o}\right] \text{ x } 100$$

Where, W_o = initial weight

W_d = weight of the sample after degradation

3.4.8 Antimicrobial activity of silver

The antimicrobial activity of silver was tested against common wound pathogens- gram positive *Staphylococcus aureus* (strain N315) and gram negative *Pseudomonas aeruginosa* (strain PA01). Colony plates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were cultured from the respective bacterial strains. 1 L of Luria agar was prepared containing 10 g of Tryptone, 5 g of yeast extract, 10 g of NaCl and agar to a final concentration of 1.5%. All the components were dissolved in 1 L of DI water. The medium was autoclaved at 121° C for 15 to 20 min and poured onto sterile petri plates and allowed to dry. Bacterial culture was inoculated using 1 colony in 3-4 ml of SWF and incubated at 37° C overnight. 10 fold serial dilutions of the pure bacterial culture were made in SWF and 10^5 dilution repeats were prepared in test tubes. 2.5 cm x 2.5 cm sample taken out from each scaffold were inserted into the 10^5 dilution test tubes and incubated at 37° C.

One test tube containing no scaffold was used as control. 100 µl (0.1 ml) of aliquot was taken out from each of the test tubes at 4 h, 24 h and 48 h and plated on luria agar plates. The plates were incubated at 37° C in the incubator overnight and observed for any bacterial growth thereafter. After incubation, the number of colonies present was counted and colony forming units/ml (cfu/ml) was reported.

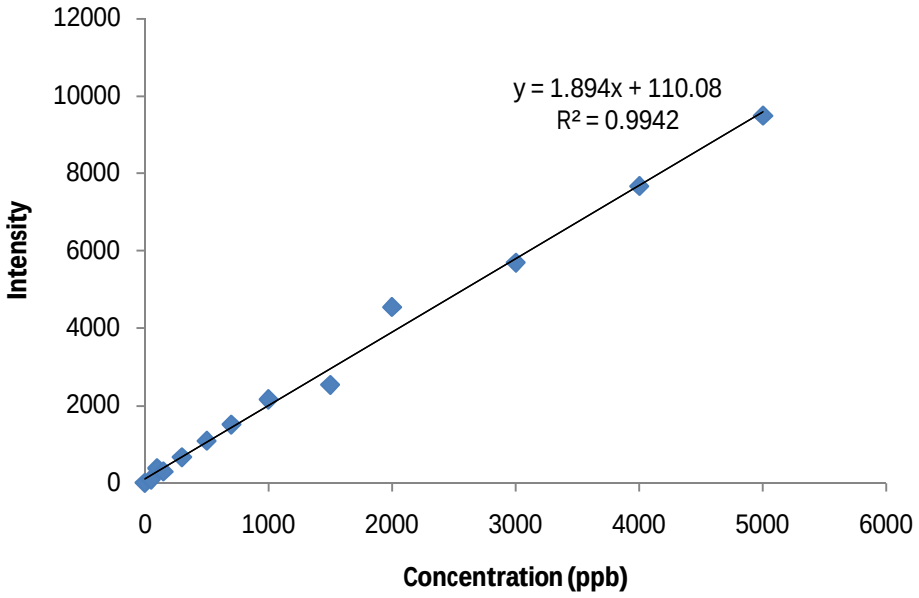
3.4.9 Silver release studies

The silver release from the scaffolds was studied in PBS. 2.5 cm x 2.5 cm of samples were taken out from the scaffolds, weighed and immersed into a capped glass vial containing 20 ml (0.02 L) of PBS. The glass vial was kept on a stir plate and the temperature was maintained at 37°C. At pre-determined time points: 1 h, 2 h, 3 h, 4 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h, 192 h, 216 h, 240 h and 264 h, 10 ml (0.01 L) of PBS was transferred to a capped tube for silver content analysis. 10 ml of fresh PBS was added to the vial to maintain the volume of the medium for continuous observation. Silver content was analyzed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES/ ICP Varian Vista MPX). ICP-OES is a technique used for analysis of trace metals present in a sample. The presence of the metals is detected by electromagnetic radiation emitted by excited atoms or ions, at a wavelength characteristic to a particular metal (Mermet 2005; Stefánsson et al. 2007). The concentration of the metal can be calculated by the intensity of the emission.

Different concentrations of aqueous silver standards were prepared from a stock solution of 1000 ppm (mg/L) silver standard. The intensity values of the known concentration of silver standards and the aliquots were recorded by ICP-OES. The calibration curve of silver (Figure 8) was used as a reference to calculate the concentration of silver in each of the aliquots. The concentration of silver in each aliquot ($[\text{concentration}]_{to}$) was obtained in the units of parts per billion (ppb or µg/L). The amount released at each time point was calculated as follows:

$$\%\ \text{Cumulative amount released (t)} = \left[\frac{[\text{concentration}]_t \text{ x } 0.02\ \text{L} + [\text{concentration}]_{to} \text{ x } 0.01\ \text{L}}{\text{Initial amount of silver in the sample (µg)}}\right] \text{x } 100$$

Figure 8: Standard curve for silver



3.4.10 Statistical analysis

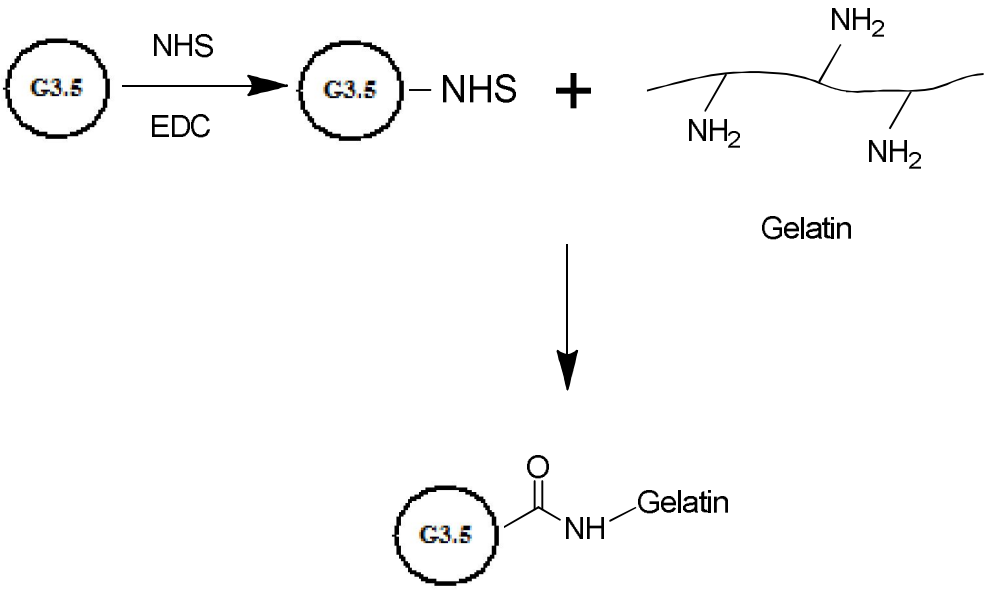
Statistical analysis was performed on mean scaffold fiber diameters, tensile testing values, porosity, permeability, pore size, swelling ratio (%) and degradation. All statistical analysis was based on one way analysis of variance (ANOVA) and Tukey's test for significance, performed on Minitab statistical software. A p-value less than 0.05 was considered statistically significant. Graphical representations of mean data were constructed with Microsoft Excel 2007 with error bars representing standard deviations.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Preparation and characterization of gelatin-dendrimer conjugates

Coupling of G3.5 to gelatin was based on EDC/NHS chemistry and the reaction mechanism is shown in Figure 9. EDC along with NHS activated the terminal carboxyl groups of G3.5. The carboxyl groups and amine groups form an amide bond, hence coupling dendrimer to the gelatin backbone.

Figure 9: Reaction mechanism- conjugation of dendrimer to gelatin



The conjugation of gelatin and G3.5 was confirmed by the ninhydrin assay. Ninhydrin produces a chromophore when mixed with amine containing compounds and its presence can be detected by measuring the absorbance value by UV-Vis spectrophotometer at a wavelength of 570 nm. As shown in the standard curve of gelatin (Figure 7), 1 mg/ml of pure gelatin mixed with 1 ml of ninhydrin was found to have an absorbance value of 0.009 whereas 1 mg/ml of G3.5-gelatin mixed with 1 ml of ninhydrin solution gave an absorbance value of 0.002. The reduced absorbance was attributed to the decrease in the number of free amine groups and hence confirmed the conjugation of dendrimer to gelatin.

4.2 Morphology, fiber diameter and mechanical properties

Electrospinning technique for fabrication of nanofiber scaffolds is gaining popularity due to its simplicity and ease of use (Kumbar et al. 2008). Electrospun scaffolds exhibit similarity in morphology to natural extra-cellular matrix (ECM), which is beneficial for tissue growth. Electrospinning can produce randomly oriented or aligned, continuous fibers which have high porosity and high surface area (Sill and von Recum 2008).

Pure gelatin alone or with gelatin-dendrimer conjugates was electrospun to form nanofiber scaffolds. The scaffolds were further crosslinked to improve their structural stability. The data shown and discussed in this chapter are based on non-crosslinked scaffolds and scaffolds crosslinked by the solution method. The structure, morphology, mechanical properties and swelling ability of the scaffolds were evaluated. Different concentrations of silver acetate were incorporated in the scaffolds to determine the antimicrobial efficacy and silver release kinetics. SEM images of non-crosslinked scaffolds and scaffolds crosslinked by the solution method are shown in Figure 10 and Figure 11, respectively. It is observed that the scaffolds crosslinked by the solution method retained the nanofiber structure. There are many factors that affect the fiber diameter and morphology, such as concentration of polymer, viscosity, voltage applied, diameter of the needle and the rate at which the polymer solution is delivered (Lee et al. 2008). The fiber diameter of the non-crosslinked scaffolds ranged from 3.15 to 5.88 μm and that of the scaffolds crosslinked by the solution method ranged from 2.64 to 6.98 μm. A graphical representation of the data is shown in Figure 12. Also, statistical differences were analyzed using ANOVA in Minitab statistical software and the data is shown in Appendix A and Appendix B. It was observed that for both non-crosslinked and crosslinked scaffolds by the solution method, the fiber diameter of the gelatin-dendrimer scaffolds (S5, S6, S7, S8) was larger than that of the scaffolds containing gelatin only (S1, S2, S3, S4). Also it was observed that as the silver concentration in the gelatin scaffolds decreased, the fiber diameter decreased but for the gelatin-dendrimer scaffolds as the silver concentration in the gelatin scaffolds increased. Half generation PAMAM dendrimers have a negative charge which could also have an influence on the electric field during electrospinning, thereby affecting the fiber diameter.

Figure 10: SEM images of the non-crosslinked scaffolds (The white block arrows indicate the notation of the scale bar of 10 μm)

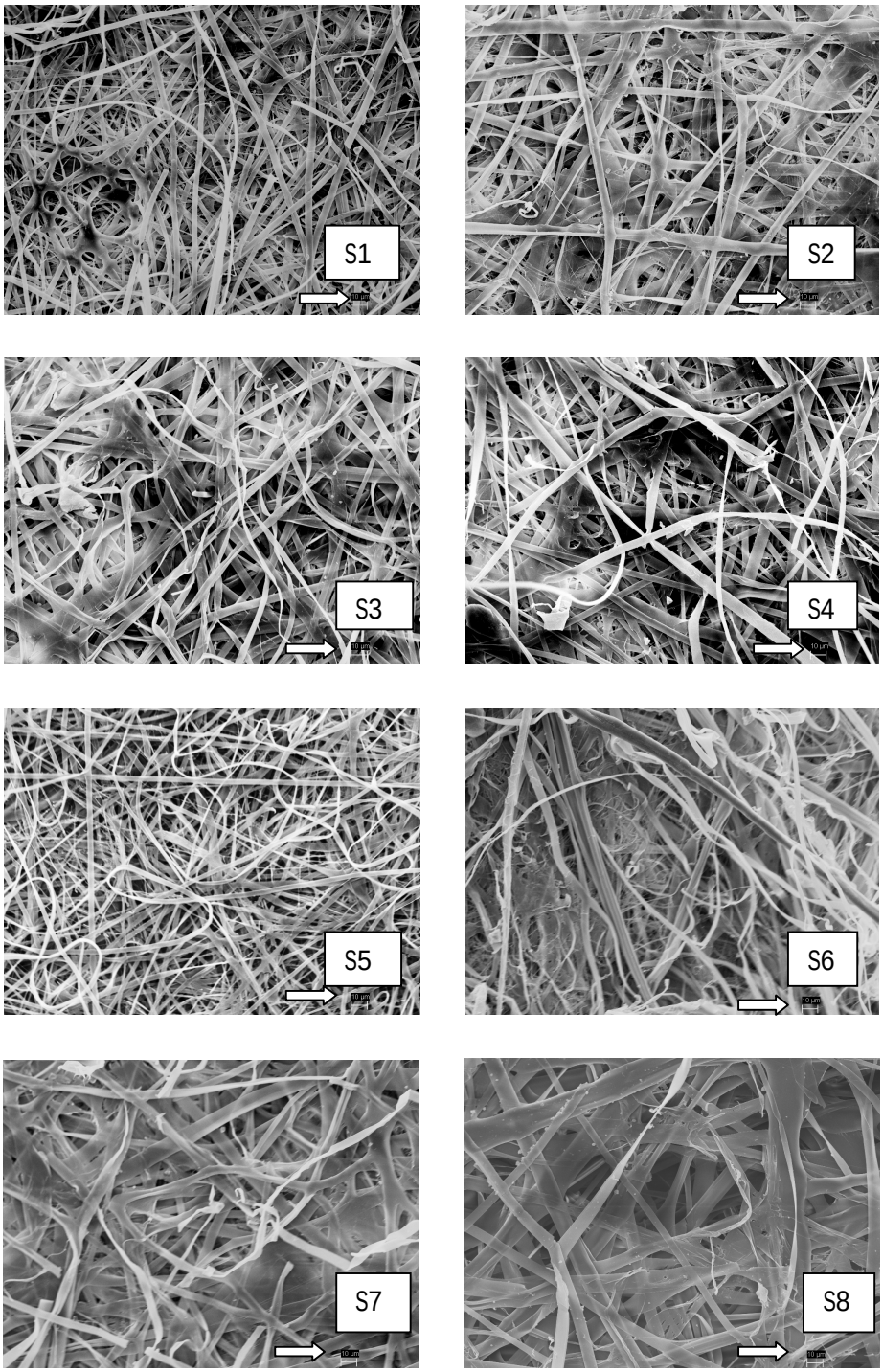


Figure 11: SEM images of the scaffolds crosslinked by the solution method (The white block arrows indicate the notation of the scale bar of 10 μm)

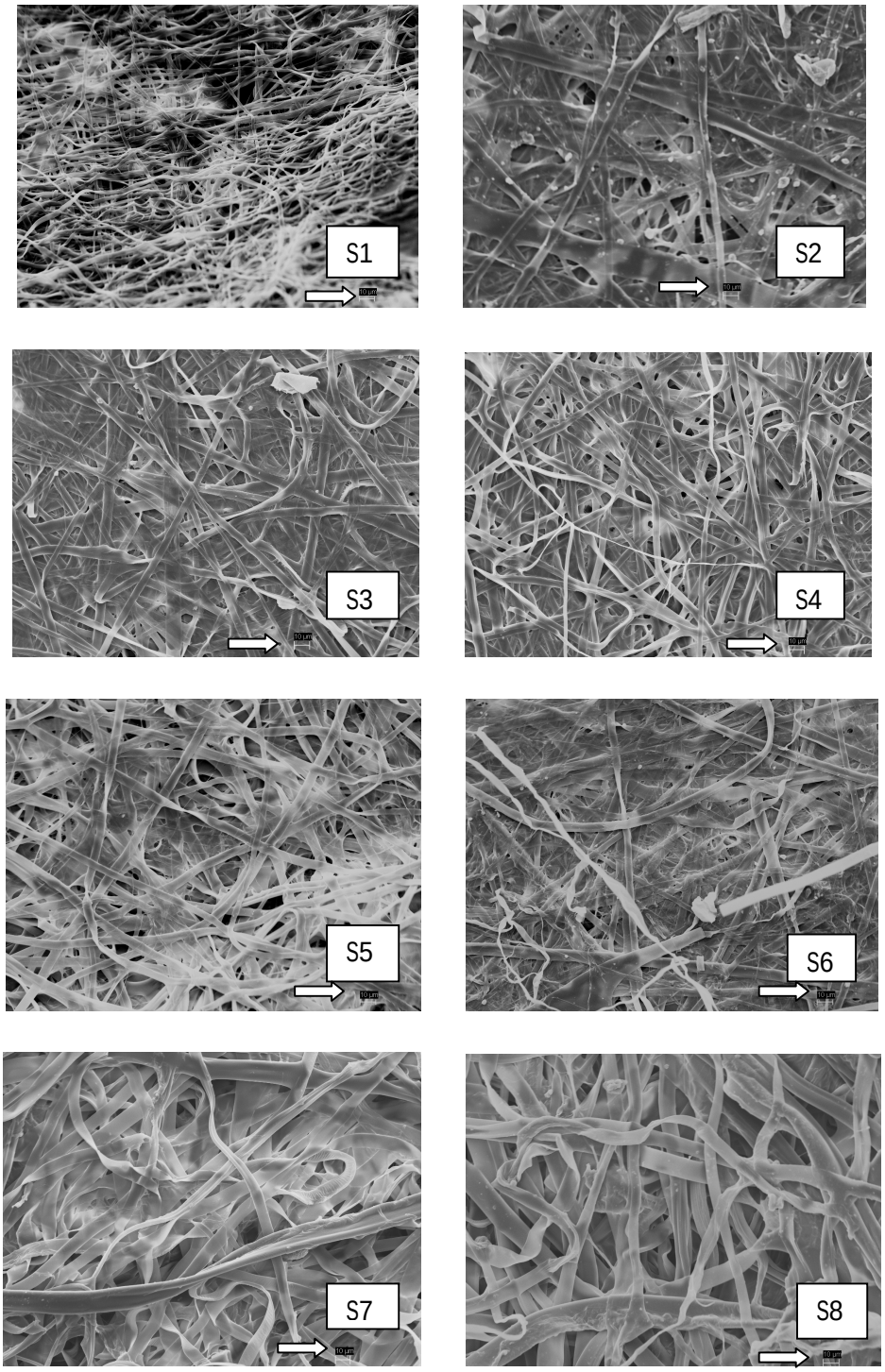
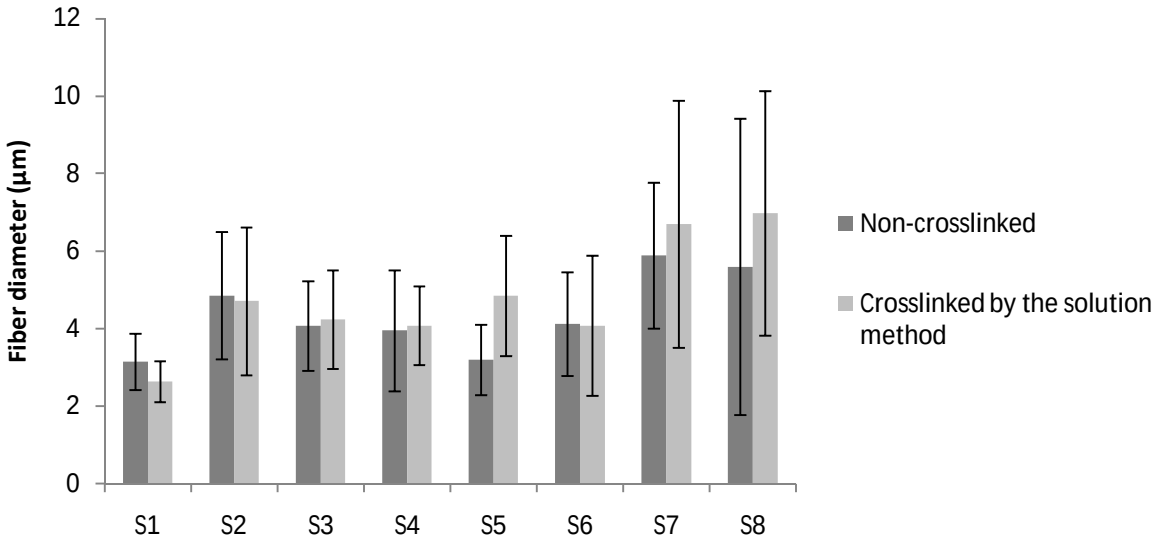


Figure 12: Fiber diameter of scaffolds (non-crosslinked and crosslinked by solution method)



Mechanical properties of a scaffold need to be evaluated for durability, stress resistance, flexibility and elasticity (Boateng et al. 2008). Peak stress, strain at break, energy to break and modulus of all scaffolds were determined by MTS Bionix and Testworks4 software. Tensile strength is the maximum stress a scaffold can withstand before breaking and determines the hardness of the scaffold. It also depends on the type and amount of polymer in the scaffold (Boateng et al. 2008). Strain at break describes the ductility and brittleness of the scaffold and also tells about the elongation of the scaffold at breaking point (Boateng et al. 2008). The mechanical properties and fiber diameter of the non-crosslinked and crosslinked scaffolds by the solution method are shown in Table 5 and Table 6, respectively; and a graphical representation of stress, strain and modulus data is shown in Figure 13. The mean stress of the non-crosslinked scaffolds ranged from 1.063 to 2.087 MPa (Appendix B) and that of crosslinked scaffolds by the solution method ranged from 0.692 to 3.125 MPa with a pooled standard deviation of 0.8616 (Appendix A). Mean stress for crosslinked scaffolds by the solution method is statistically higher in the S1 scaffold type which contained only gelatin. It was observed that the stress values were higher for the non-crosslinked scaffolds as compared to the crosslinked ones except for scaffold S1. This behaviour was also observed in the modulus results. The scaffolds containing silver displayed higher stress values than scaffolds without silver. The mean strain for the non crosslinked scaffolds ranged from 0.015 to 0.040 mm/mm and for the crosslinked scaffolds by

the solution method ranged from 0.020 to 0.067 mm/mm. The strain values for crosslinked scaffolds were higher than the non-crosslinked scaffolds except for scaffold S2.

Further characterization to determine the porosity, permeability, swelling ratio, antimicrobial activity and silver release kinetics was performed only on scaffolds crosslinked by the solution method since they could retain their stability in aqueous medium.

Table 5 : Tensile studies and fiber diameter of the non-crosslinked scaffolds

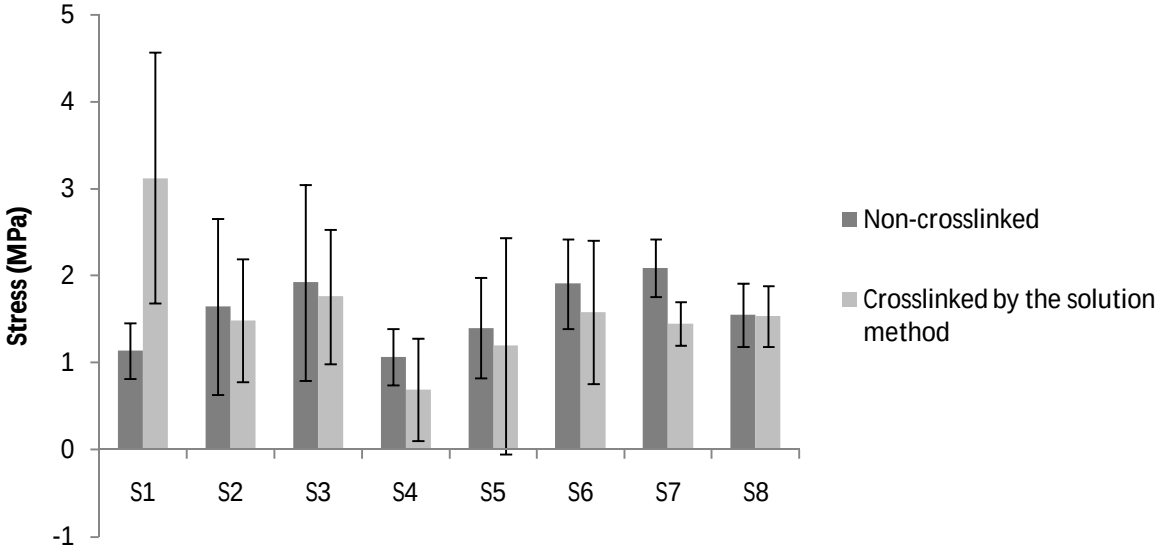
Scaffold	Fiber diameter (μm)	Peak stress (MPa)	Modulus (MPa)	Strain at break (mm/mm)	Energy to break (N*mm)
S1	3.15 ± 0.72	1.136 ± 0.319	47.134 ± 6.463	0.028 ± 0.007	0.203 ± 0.097
S2	4.85 ± 1.64	1.646 ± 1.016	93.636 ± 46.672	0.040 ± 0.038	0.272 ± 0.286
S3	4.07 ± 1.15	1.922 ± 1.128	99.276 ± 45.636	0.022 ± 0.007	0.13 ± 0.050
S4	3.95 ± 1.96	1.063 ± 0.323	64.841 ± 15.302	0.018 ± 0.007	0.107 ± 0.064
S5	3.20 ± 0.90	1.399 ± 0.578	80.525 ± 20.326	0.022 ± 0.005	0.183 ± 0.090
S6	4.12 ± 1.34	1.908 ± 0.519	66.580 ± 18.809	0.037 ± 0.009	0.476 ± 0.173
S7	5.88 ± 1.88	2.087 ± 0.335	104.835 ± 18.741	0.027 ± 0.006	0.332 ± 0.174
S8	5.59 ± 3.83	1.549 ± 0.363	100.319 ± 27.641	0.015 ± 0.005	0.137 ± 0.079

Table 6: Tensile studies and fiber diameter of the scaffolds crosslinked by the solution method

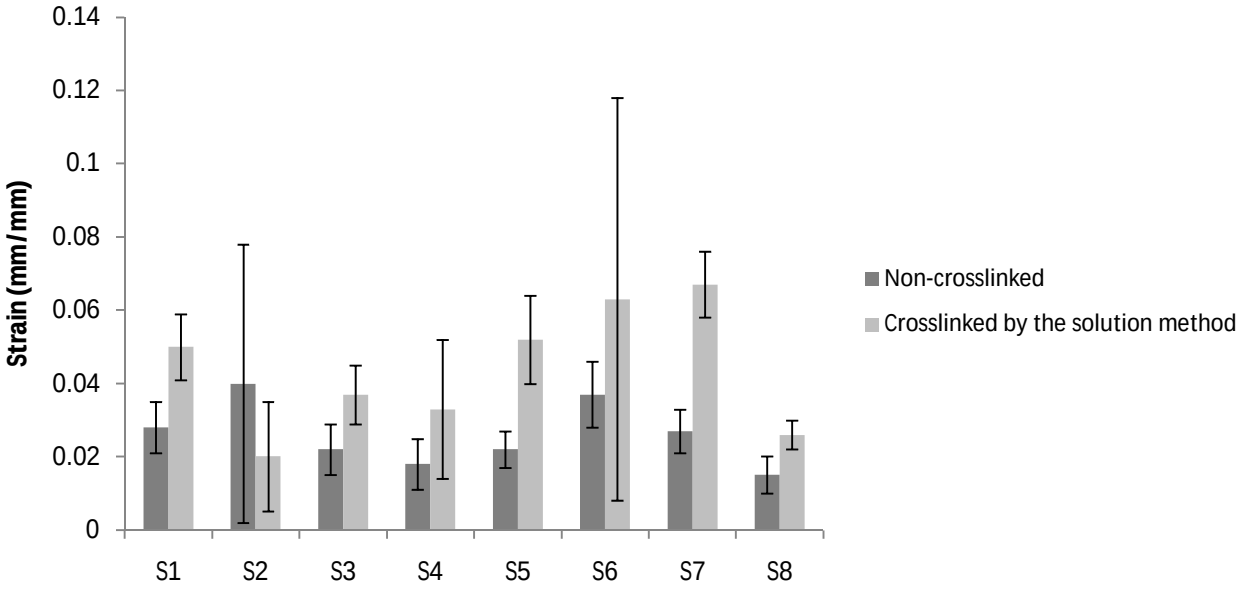
Scaffold	Fiber diameter (μm)	Peak stress (MPa)	Modulus (MPa)	Strain at break (mm/mm)	Energy to break (N*mm)
S1	2.64 ± 0.53	3.125 ± 1.443	80.835 ± 24.326	0.050 ± 0.009	0.060 ± 0.366
S2	4.71 ± 1.90	1.485 ± 0.703	92.709 ± 35.127	0.020 ± 0.015	0.011 ± 0.075
S3	4.24 ± 1.27	1.760 ± 0.774	58.330 ± 20.242	0.037 ± 0.008	0.306 ± 0.205
S4	4.08 ± 1.02	0.692 ± 0.587	38.271 ± 12.854	0.033 ± 0.019	0.115 ± 0.140
S5	4.85 ± 1.55	1.196 ± 1.246	53.443 ± 53.337	0.052 ± 0.012	0.278 ± 0.319
S6	4.08 ± 1.80	1.581 ± 0.826	59.370 ± 27.612	0.063 ± 0.055	0.422 ± 0.203
S7	6.7 ± 3.19	1.447 ± 0.250	33.804 ± 8.913	0.067 ± 0.009	0.646 ± 0.102
S8	6.98 ± 3.16	1.533 ± 0.348	69.395 ± 21.298	0.026 ± 0.004	0.192 ± 0.062

Figure 13: Graphical representation of stress, strain and modulus (A: Stress, B: Strain, C: Modulus)

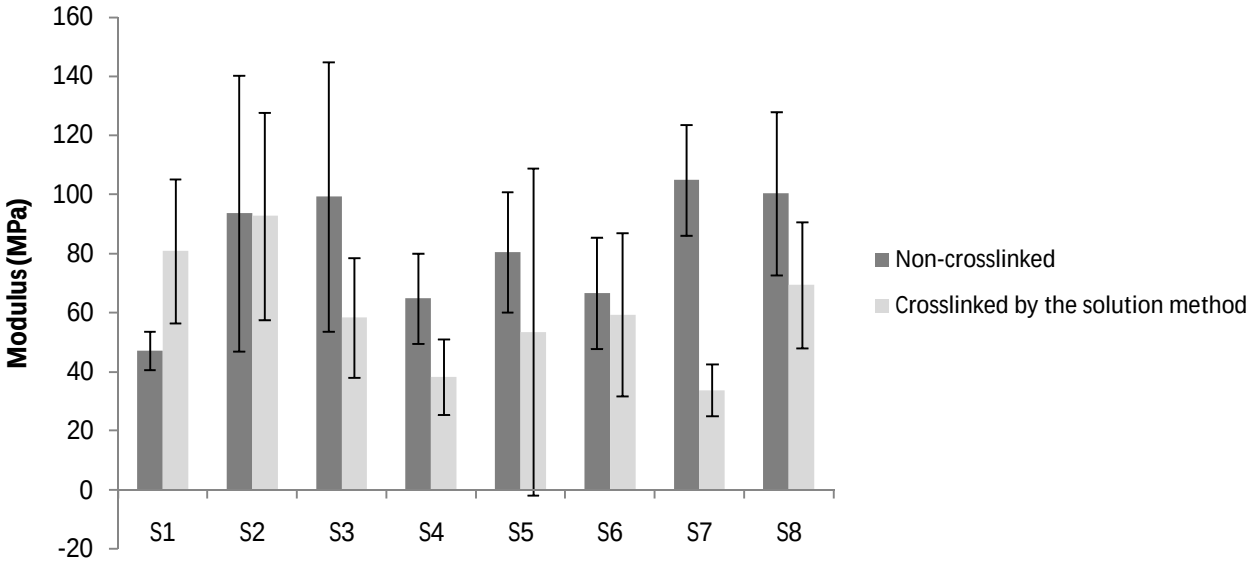
A



B



C



4.3 Scaffold porosity, permeability, swelling and degradation studies

The porosity, permeability and swelling ability of the scaffolds were evaluated to verify their capability for medium exchange. Porosity is the measure of void space within the scaffolds. The graphical data for porosity is shown in Figure 14 and the statistical analysis for the data is shown in Appendix A. The porosity of the scaffolds crosslinked by the solution method ranged from 67.56 to 90.42% which is suitable as a tissue engineered scaffold for adequate moisture and oxygen exchange to underlying cells (Freed et al. 1994). The porosity of scaffold S8 was significantly higher than the porosity of scaffolds S3, S4 and S5 and highest among all the porosity values. Permeability is the measure of the ease of flow of fluid through the scaffold. Permeability ranged from 0.1673 to 2.428 Darcy and is depicted in Figure 15. Also the statistical analysis for permeability and pore size data is shown in Appendix A. It is observed that the gelatin-dendrimer scaffolds containing silver had lower permeability values as compared to the gelatin scaffolds containing silver but the statistical analysis shows that the data is not statistically significant. This lower permeability values of the gelatin-dendrimer scaffolds containing silver may be due to their higher fiber diameter. Pore size of the scaffolds show a similar trend to what was found in permeability as shown in Figure 16.

Figure 14: Porosity of the scaffolds crosslinked by the solution method

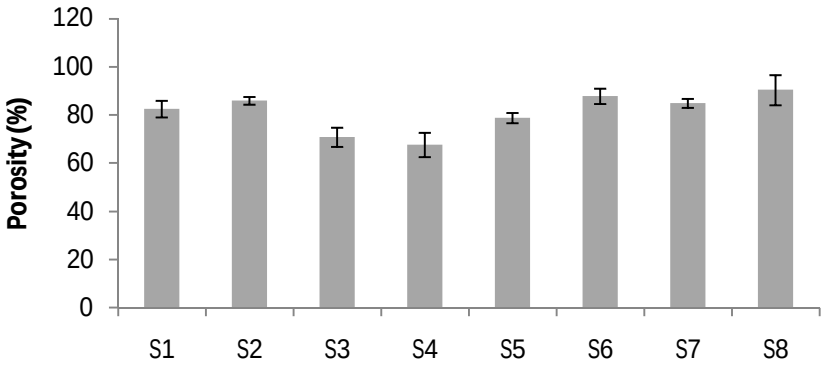


Figure 15: Permeability of the scaffolds crosslinked by the solution method

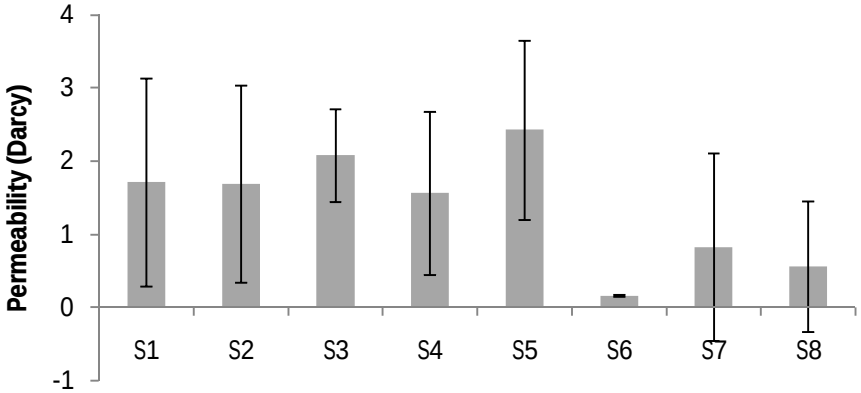
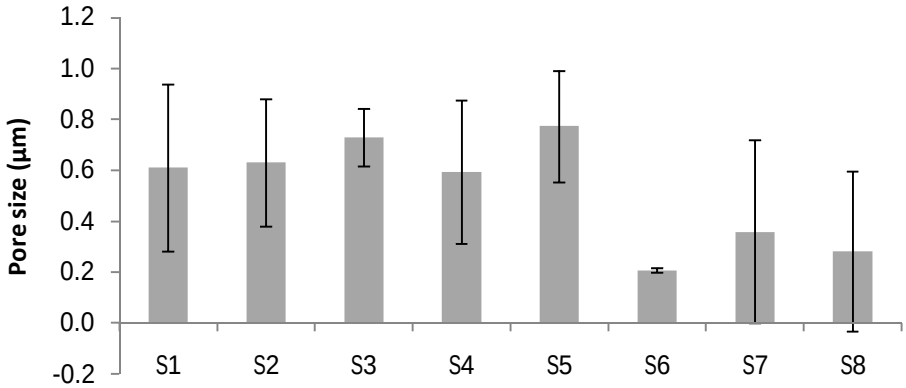
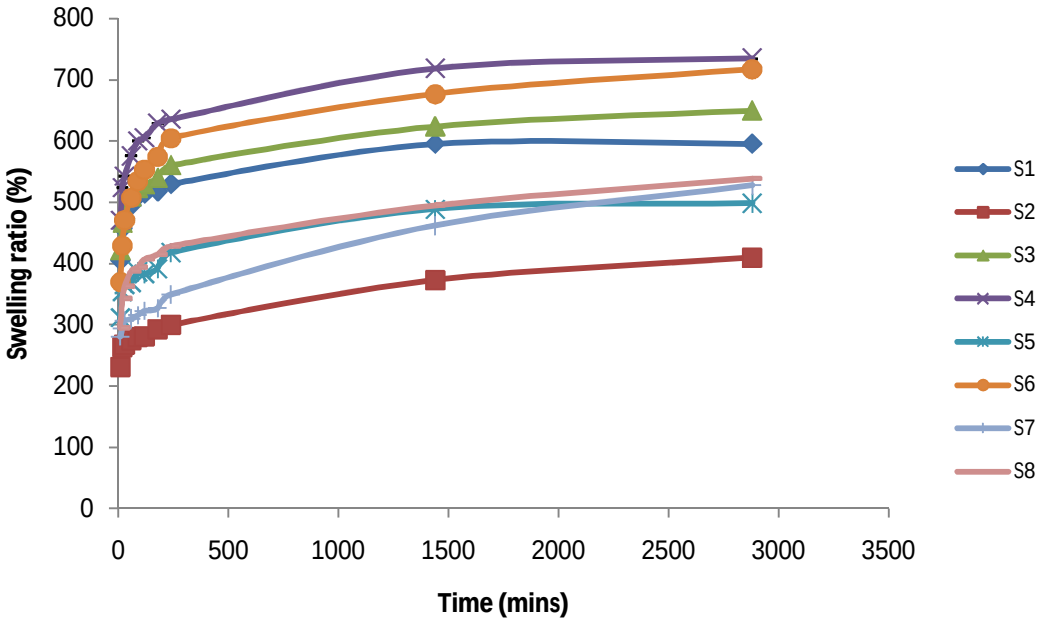


Figure 16: Pore size of the scaffolds crosslinked by the solution method



Adsorption and swelling studies were done to determine the swelling capacity of the scaffolds. Wounds that have extensive bleeding and heavy amounts of exudate require dressings to absorb quickly and maintain a clean environment at the wound site. The swelling studies were carried out in the SWF to replicate the clinical conditions. A graphical representation of the data is shown in Figure 17 and a statistical analysis of the swelling ratio values at 48 h is shown in Appendix A. All the scaffolds demonstrated a good swelling and absorbing capacity. The swelling ratio varied from 415 to 626 % at the end of 48 h (2880 min). It is observed that scaffold S4 had the highest swelling ratio.

Figure 17: Swelling of the scaffolds crosslinked by the solution method (in SWF at room temperature)

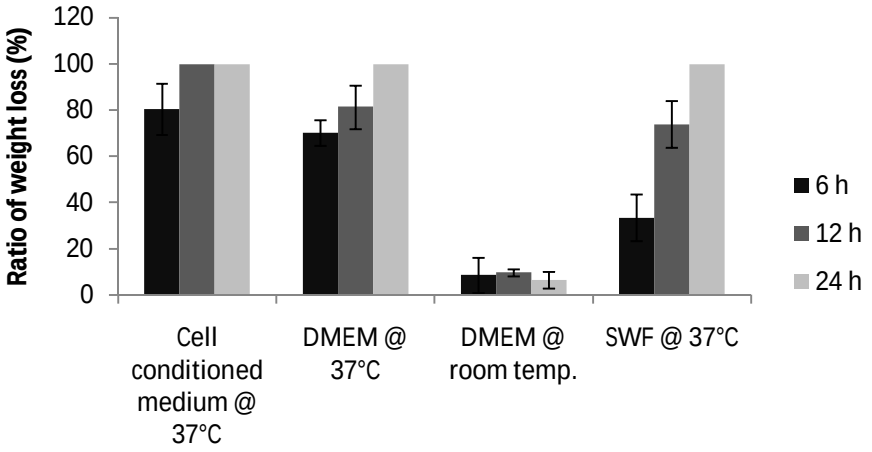


Gelatin is easily soluble and therefore scaffolds containing gelatin need to be evaluated for degradation. The scaffolds without silver (S1 and S5) were evaluated for degradation studies to observe the effect of temperature and other factors, if any, on the rate of degradation of the scaffold. It was observed that temperature did play a role in the rate of degradation as shown in Figure 18 for scaffold S1 and S5. A statistical analysis of the data is shown in Appendix A. For both the scaffolds, the weight loss (%) was significantly lower when tested at room temperature as compared to incubation at 37°C. The type of medium also affected the rate of degradation.

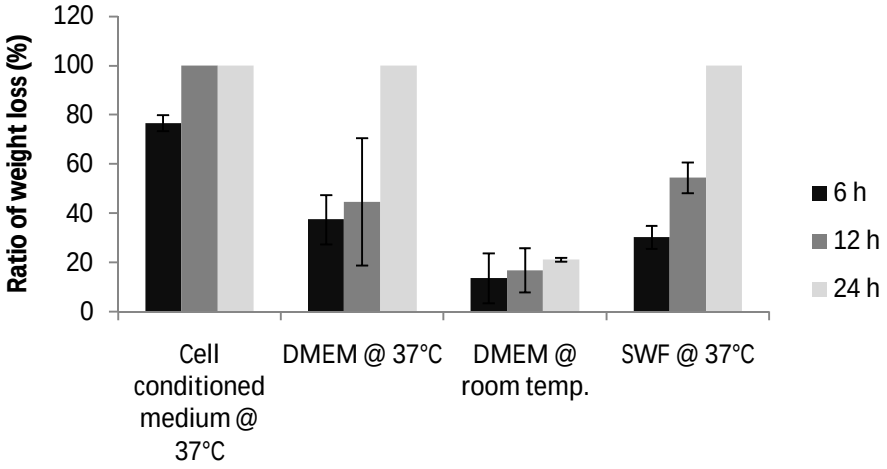
Both the scaffolds degraded completely at the end of 24 h in all media at 37°C, with the weight loss being highest during incubation in cell conditioned medium. It was also observed that S5 which contained gelatin-dendrimer conjugate had lesser degradation than S1 which contained only gelatin when incubated in same media at 37°C.

Figure 18: Degradation of the scaffolds crosslinked by the solution method

Scaffold S1



Scaffold S5



4.4 Antimicrobial assay

The scaffolds crosslinked by solution method were tested for antimicrobial efficacy against two common wound pathogens, gram positive *Staphylococcus aureus* and gram negative *Pseudomonas aeruginosa*. Gram positive bacteria do not contain any outer membrane but have a thick peptidoglycan layer and stain dark blue or violet by Gram's staining. In contrast, gram negative bacteria contain an outer membrane but have a thin peptidoglycan layer and stain pink by Gram's staining. The results for colony forming units/ml and the images of the petri plates are shown in Table 7, Table 8, Figure 19 and Figure 20. It is observed that the growth of both bacteria increased in a span of 48 h for the culture test tubes with no sample (control) and those containing scaffold samples without silver (S1 and S5). For *Staphylococcus aureus*, it was observed that at the end of 4 h, bacterial growth was completely inhibited in the test tubes containing S2, S3, S5 and S6, but there was some growth in the test tubes containing S4 and S8. Overall it was observed that all the scaffold samples containing silver inhibited the growth of *Staphylococcus aureus* by the end of 48 h. For *Pseudomonas aeruginosa*, it was observed that all the scaffold samples containing silver inhibited the growth of bacteria by the end of 48 h.

Table 7: Antimicrobial activity against *Staphylococcus aureus*

Scaffold type	4 h	24 h	48 h
Control (untreated)	10×10^7 cfu/ml	24×10^7 cfu/ml	Bacterial lawn
S1	5.1×10^7 cfu/ml	6×10^7 cfu/ml	Bacterial lawn
S2	No colonies	No colonies	No colonies
S3	No colonies	No colonies	No colonies
S4	0.7×10^7 cfu/ml	No colonies	No colonies
S5	6×10^7 cfu/ml	Bacterial lawn	Bacterial lawn
S6	No colonies	No colonies	No colonies
S7	No colonies	No colonies	No colonies
S8	1.5×10^7 cfu/ml	No colonies	No colonies

Table 8: Antimicrobial activity against *Pseudomonas aeruginosa*

Sample type	4 h	24 h	48 h
Control (untreated)	Bacterial lawn	Bacterial lawn	Bacterial lawn
S1	Bacterial lawn	Bacterial lawn	Bacterial lawn
S2	No colonies	No colonies	No colonies
S3	No colonies	No colonies	No colonies
S4	No colonies	No colonies	No colonies
S5	Bacterial lawn	Bacterial lawn	Bacterial lawn
S6	No colonies	No colonies	No colonies
S7	No colonies	No colonies	No colonies
S8	No colonies	No colonies	No colonies

4.5 Silver release kinetics

Silver release kinetics were measured by means of diffusion of silver into PBS medium and analyzing the silver content by ICP-OES. A graphical representation of cumulative release of silver (%) over time is shown in Figure 21. It is observed that all the scaffolds containing silver show a similar drug release pattern over a span of 264 h (short term). Silver release was slow and a very small amount of silver was released at the end of 264 h. It is also observed that larger amount of silver is released from gelatin-dendrimer scaffolds as compared to gelatin scaffolds containing equal amounts of silver (i.e. S2 and S6, S3 and S7, S4 and S8). This may be due to the larger fiber diameter of gelatin-dendrimer scaffolds. Fibers with larger diameter have a greater surface area for diffusion. Comparison of the antimicrobial assay and silver release kinetics revealed that even a low amount of silver released could inhibit any bacterial growth by 48 h.

Figure 19: Antimicrobial activity against *Staphylococcus aureus*

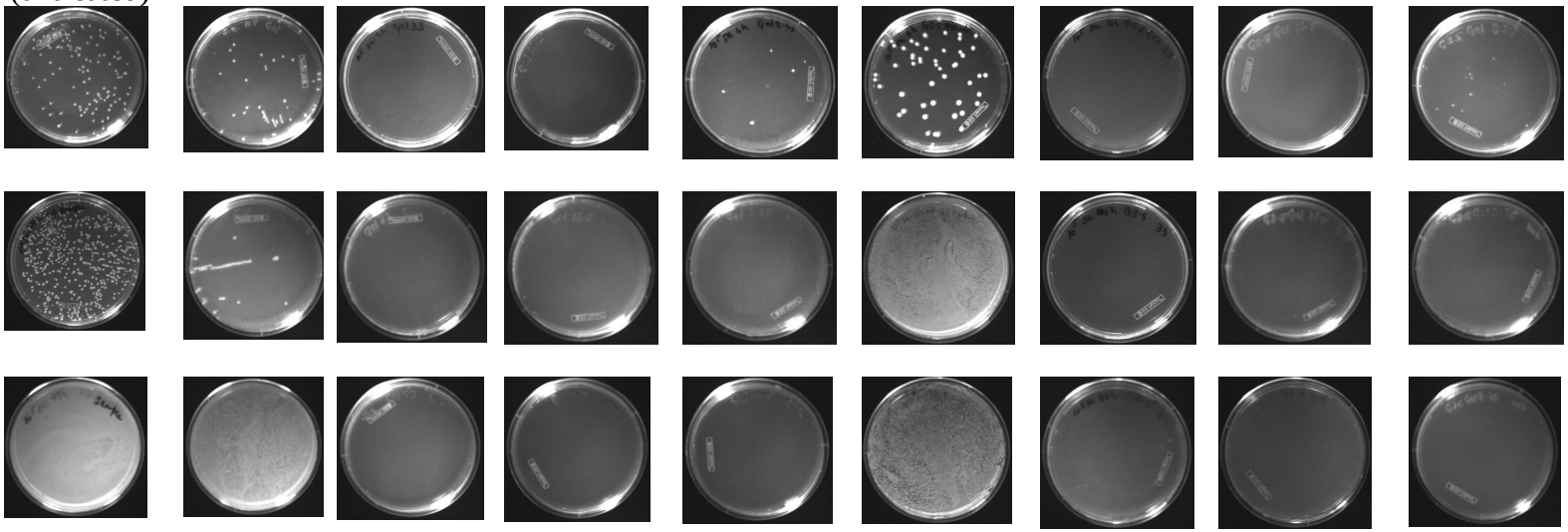


Figure 20: *Antimicrobial activity against Pseudomonas aeruginosa*

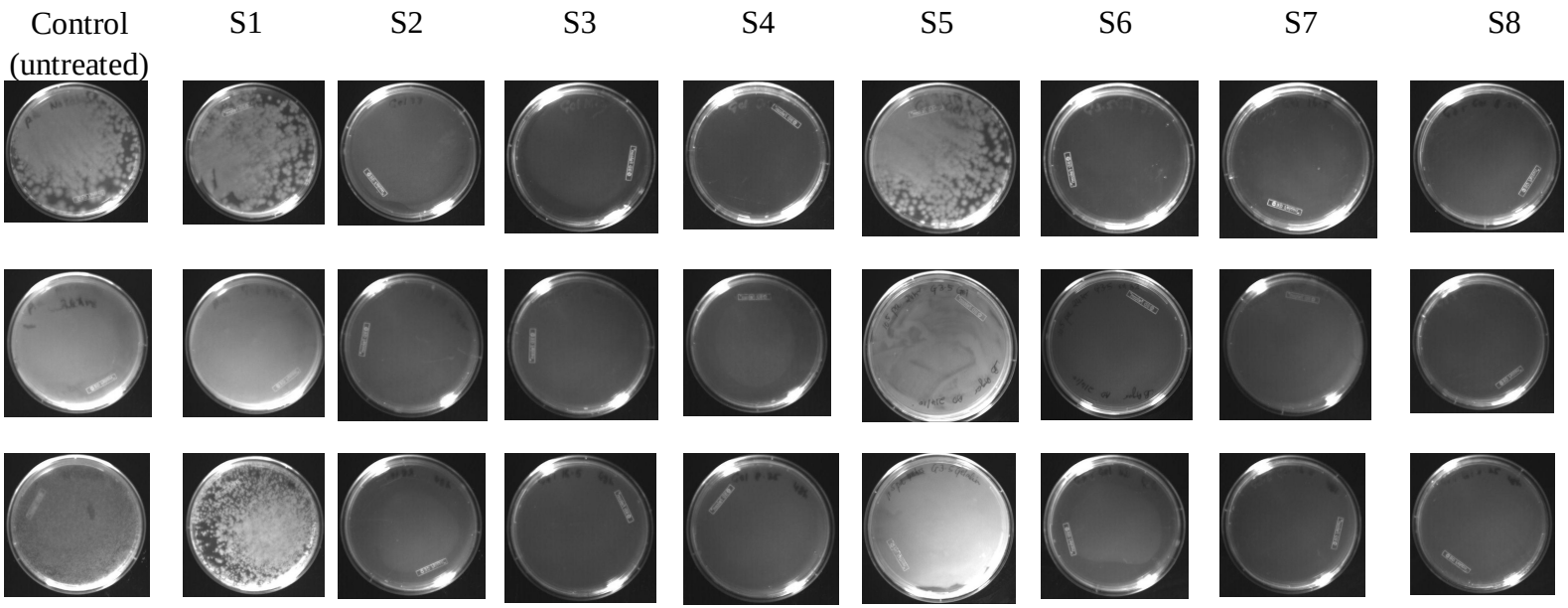
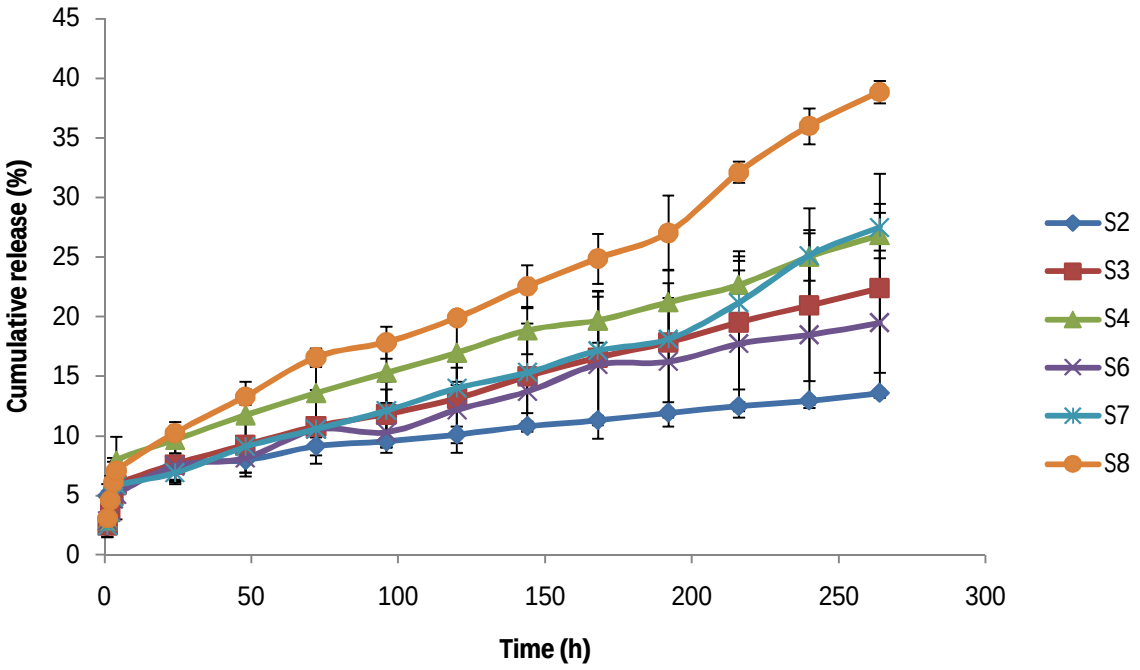


Figure 21: Silver release kinetics



4.6 Conclusions

This work demonstrated that silver containing nanofiber dressings composed of gelatin with or without gelatin-dendrimer conjugates could be fabricated by electrospinning. The scaffolds were successfully crosslinked by photo-polymerization using liquid or vapor form of PEG diacrylate for structure stability improvement. The characteristics in terms of morphology, fiber diameter, mechanical properties, porosity, permeability and swelling capability could be modulated by changing the composition of the scaffold. According to antimicrobial assay, silver-containing scaffolds could inhibit bacterial growth by 48 h. Silver could be released in a controlled manner over an extended period of time.

CHAPTER 6 SUMMARY AND FUTURE WORK

Tissue engineered scaffolds have a wide range of applications as a wound dressing, for tissue regeneration and development and as a vehicle for drug delivery. They mimic the natural extra-cellular matrix and facilitate cell growth and proliferation. We designed a novel gelatin-dendrimer nanofiber scaffold as a potential wound dressing. The scaffolds were characterized to quantify their mechanical properties, gas and nutrient flow capability, swelling capacity and drug release kinetics. The scaffolds which showed the desirable properties of a wound dressing are summarized in Table 9.

Table 9: Summary table

Properties	S1	S2	S3	S4	S5	S6	S7	S8
Stress	✓✓					✓		✓
Strain					✓	✓		
Porosity		✓				✓		✓✓
Permeability					✓			
Swelling	✓		✓	✓✓		✓		✓
Antimicrobial activity		✓✓	✓✓	✓		✓✓	✓✓	✓
Drug release						✓	✓	✓✓

The scaffolds were required to be crosslinked to acquire the properties of a tissue engineered scaffold. The scaffolds have a unique structural configuration in which covalently bound dendrimer is evenly distributed along the dimension of the nanofiber. The addition of dendrimer provides multiple functional groups for attachment of drugs and their controlled release. The scaffolds S2, S6 and S8 were highly porous in nature and the higher fiber diameter provided

greater surface area for diffusion and drug release. The scaffolds S1, S2, S4, S6 and S8 also exhibited high capacity of swelling and adsorption. The scaffolds S1 and S5 were found to be easily degradable when incubated in cell conditioned medium at 37°C. This could be due to gelatin which is easily soluble and needs higher density of crosslinking for stability. The concentration of PEG-diacrylate could be increased to improve the crosslinking density hence controlling the rate of degradation. We incorporated silver into the scaffold to determine its antimicrobial efficacy; various other antimicrobial agents can be used for future studies. The scaffolds with silver could effectively inhibit growth of bacteria by 24 h. Also the concentration of silver can be further reduced to avoid any potential accumulated toxicity. As future work, studies could be done to evaluate the effect and clearance of silver in vivo to get a better insight. The scaffolds fabricated show promise as a bioactive dressing to treat wounds. They exhibit the properties necessary to maintain a moist environment to facilitate wound healing.

The process of wound healing and wound closure involves growth factors like FGF, PDGF and TGF-β. As an extended study, growth factors like TGF-β could be incorporated into the scaffolds to increase the rate of healing and for release studies. TGF-β promotes the production of collagen framework thus helping in the proliferative phase of wound healing. Also the designed scaffold can be used to deliver other therapeutics for treating a variety of wounds, including chronic wounds, burns and skin cancer.

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APPENDICES Page

Interpretation of the statistical analysis shown in Appendix A, B and C

Analysis of Variance (ANOVA):

- $P < 0.05$ indicates that the data is statistically significant

Tukey's Pairwise Comparisons:

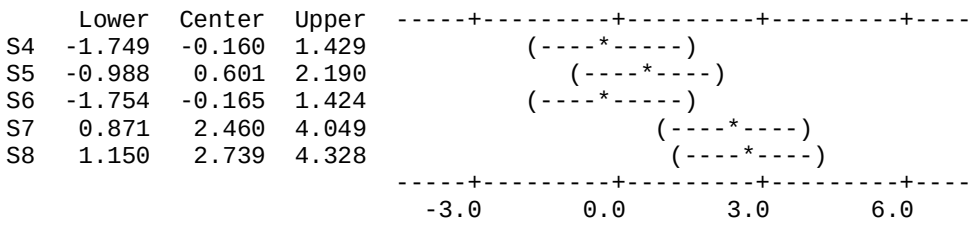
- Tukey's pairwise comparison is performed to determine if two data values is statistically different from one another
- If the confidence interval (lower to upper) between two data values does not include a zero, the difference between them is statistically significant
- If the confidence interval (lower to upper) between two data values includes a zero, the difference between them is not statistically significant

Appendix A (Data for the scaffolds crosslinked by the solution method)

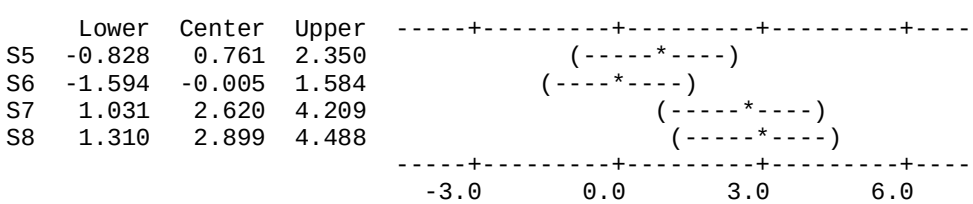
One-way ANOVA: Fiber diameter

```
Source   DF       SS     MS      F      P
Factor    7   432.43  61.78  15.29  0.000
Error   232   937.12   4.04
Total   239  1369.55

S = 2.010   R-Sq = 31.57%   R-Sq(adj) = 29.51%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level   N   Mean  StDev  -------+---------+---------+---------+--
S1     30  2.638  0.525  (----*---)
S2     30  4.714  1.902              (---*----)
S3     30  4.244  1.273           (----*----)
S4     30  4.084  1.016         (----*----)
S5     30  4.845  1.547              (----*----)
S6     30  4.079  1.800         (----*----)
S7     30  6.704  3.189                        (----*----)
S8     30  6.983  3.157                          (----*---)
                         -------+---------+---------+---------+--
                              3.0       4.5       6.0       7.5

Pooled StDev = 2.010


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.75%


S1 subtracted from:

     Lower  Center  Upper  -----+---------+---------+---------+----
S2   0.486   2.075  3.664                  (----*----)
S3   0.017   1.606  3.195               (----*-----)
S4  -0.143   1.446  3.035               (----*----)
S5   0.618   2.207  3.796                 (----*-----)
S6  -0.148   1.441  3.030               (----*----)
S7   2.477   4.066  5.655                       (-----*----)
S8   2.756   4.345  5.934                        (----*-----)
                         -----+---------+---------+---------+----
                           -3.0       0.0       3.0       6.0


S2 subtracted from:

     Lower  Center  Upper  -----+---------+---------+---------+----
S3  -2.058  -0.469  1.120         (----*-----)
S4  -2.219  -0.630  0.959         (----*----)
S5  -1.458   0.131  1.720           (----*-----)
S6  -2.224  -0.635  0.954         (----*----)
S7   0.401   1.990  3.579                 (-----*----)
S8   0.681   2.270  3.859                  (-----*----)
                         -----+---------+---------+---------+----
                           -3.0       0.0       3.0       6.0
```

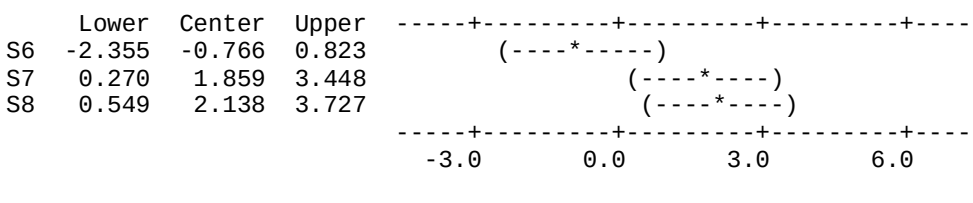
S3 subtracted from:



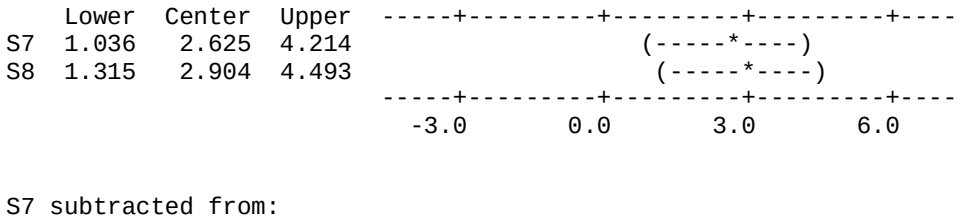
S4 subtracted from:



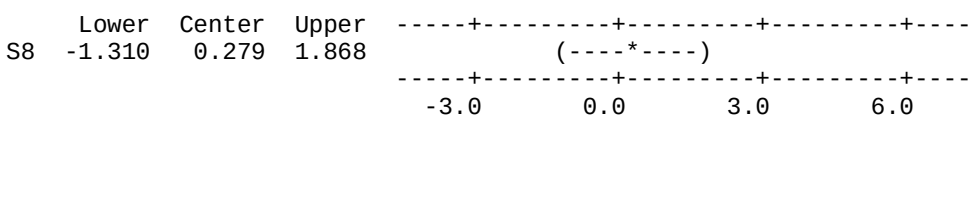
S5 subtracted from:



S6 subtracted from:



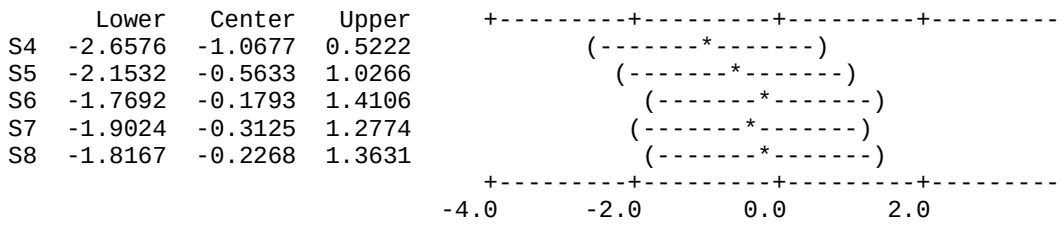
S7 subtracted from:



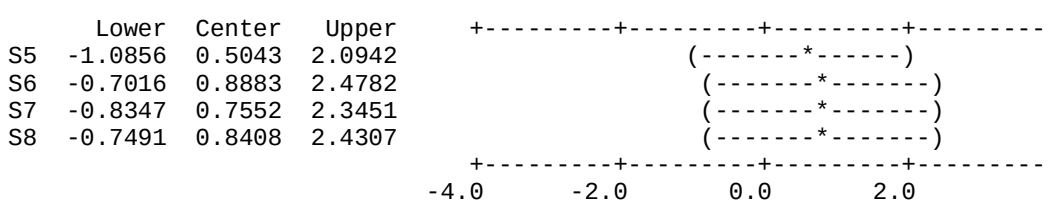
One-way ANOVA: Stress

```
Source  DF      SS     MS     F      P
Factor   7  20.277  2.897  3.90  0.002
Error   40  29.695  0.742
Total   47  49.972

S = 0.8616   R-Sq = 40.58%   R-Sq(adj) = 30.18%


                          Individual 95% CIs For Mean Based on Pooled StDev
Level  N    Mean   StDev    +---------+---------+---------+---------
S1     6  3.1250  1.4434                          (------*------)
S2     6  1.4848  0.7033             (------*------)
S3     6  1.7598  0.7738               (-------*------)
S4     6  0.6922  0.5866    (------*------)
S5     6  1.1965  1.2460         (------*------)
S6     6  1.5805  0.8256            (------*------)
S7     6  1.4473  0.2503          (------*-------)
S8     6  1.5330  0.3484            (------*------)
                            +---------+---------+---------+---------
                           0.0       1.0       2.0       3.0

Pooled StDev = 0.8616


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.73%


S1 subtracted from:

      Lower   Center    Upper     +---------+---------+---------+---------
S2  -3.2301  -1.6402  -0.0503         (-------*-------)
S3  -2.9551  -1.3652   0.2247          (-------*-------)
S4  -4.0227  -2.4328  -0.8429     (-------*-------)
S5  -3.5184  -1.9285  -0.3386       (-------*-------)
S6  -3.1344  -1.5445   0.0454         (-------*-------)
S7  -3.2676  -1.6777  -0.0878         (-------*-------)
S8  -3.1819  -1.5920  -0.0021         (-------*-------)
                              +---------+---------+---------+---------
                           -4.0      -2.0       0.0       2.0


S2 subtracted from:

      Lower   Center   Upper     +---------+---------+---------+---------
S3  -1.3149   0.2750  1.8649                (-------*-------)
S4  -2.3826  -0.7927  0.7972          (-------*-------)
S5  -1.8782  -0.2883  1.3016             (-------*-------)
S6  -1.4942   0.0957  1.6856               (------*-------)
S7  -1.6274  -0.0375  1.5524              (-------*-------)
S8  -1.5417   0.0482  1.6381              (-------*-------)
                              +---------+---------+---------+---------
                           -4.0      -2.0       0.0       2.0
```

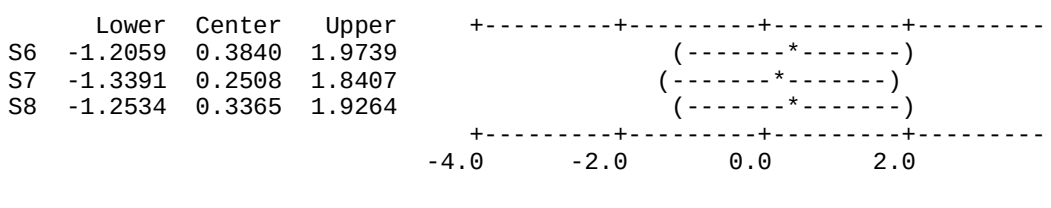
S3 subtracted from:



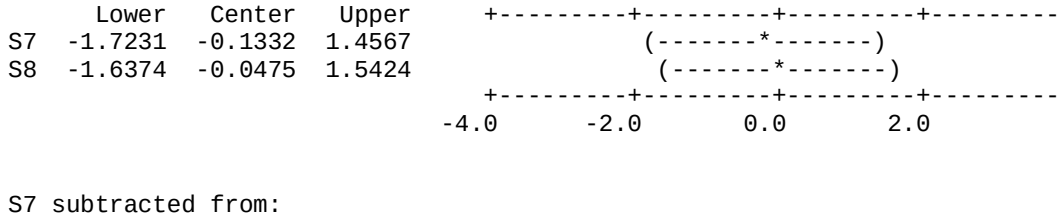
S4 subtracted from:



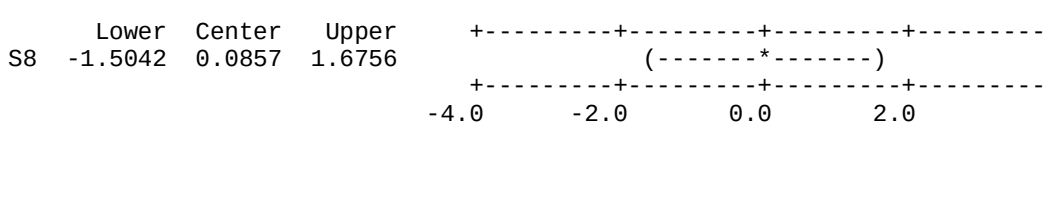
S5 subtracted from:



S6 subtracted from:



S7 subtracted from:



One-way ANOVA: Strain

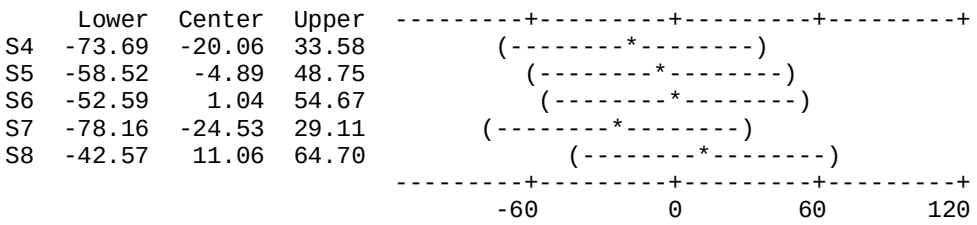
```
Source  DF        SS        MS     F      P
Factor   7  0.012315  0.001759  3.53  0.005
Error   40  0.019907  0.000498
Total   47  0.032222

S = 0.02231   R-Sq = 38.22%   R-Sq(adj) = 27.41%


                            Individual 95% CIs For Mean Based on Pooled StDev
Level  N     Mean    StDev  ---------+---------+---------+---------+
S1     6  0.04967  0.00937             (------*------)
S2     6  0.01983  0.01508  (------*------)
S3     6  0.03700  0.00780       (-------*------)
S4     6  0.03267  0.01933      (------*------)
S5     6  0.05167  0.01147             (-------*------)
S6     6  0.06317  0.05478                  (------*-------)
S7     6  0.06700  0.00897                   (-------*------)
S8     6  0.02650  0.00432    (-------*------)
                            ---------+---------+---------+---------+
                                   0.025     0.050     0.075     0.100

Pooled StDev = 0.02231


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.73%


S1 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S2  -0.07100  -0.02983  0.01133      (-------*-------)
S3  -0.05383  -0.01267  0.02850         (-------*--------)
S4  -0.05817  -0.01700  0.02417        (--------*-------)
S5  -0.03917   0.00200  0.04317            (-------*--------)
S6  -0.02767   0.01350  0.05467              (--------*-------)
S7  -0.02383   0.01733  0.05850               (-------*--------)
S8  -0.06433  -0.02317  0.01800       (-------*--------)
                                  --------+---------+---------+---------+-
                                       -0.050     0.000     0.050     0.100


S2 subtracted from:

       Lower   Center    Upper  --------+---------+---------+---------+-
S3  -0.02400  0.01717  0.05833              (-------*--------)
S4  -0.02833  0.01283  0.05400             (--------*-------)
S5  -0.00933  0.03183  0.07300                 (-------*--------)
S6   0.00217  0.04333  0.08450                   (--------*-------)
S7   0.00600  0.04717  0.08833                    (-------*--------)
S8  -0.03450  0.00667  0.04783            (-------*--------)
                                  --------+---------+---------+---------+-
                                       -0.050     0.000     0.050     0.100
```

```
S3 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S4  -0.04550  -0.00433  0.03683           (-------*-------)
S5  -0.02650   0.01467  0.05583               (-------*-------)
S6  -0.01500   0.02617  0.06733                 (-------*-------)
S7  -0.01117   0.03000  0.07117                  (-------*-------)
S8  -0.05167  -0.01050  0.03067          (-------*-------)
                                  --------+---------+---------+---------+-
                                       -0.050     0.000     0.050     0.100


S4 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S5  -0.02217   0.01900  0.06017               (-------*-------)
S6  -0.01067   0.03050  0.07167                 (-------*-------)
S7  -0.00683   0.03433  0.07550                  (-------*-------)
S8  -0.04733  -0.00617  0.03500           (-------*-------)
                                  --------+---------+---------+---------+-
                                       -0.050     0.000     0.050     0.100


S5 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S6  -0.02967   0.01150  0.05267             (-------*--------)
S7  -0.02583   0.01533  0.05650              (-------*-------)
S8  -0.06633  -0.02517  0.01600       (-------*-------)
                                  --------+---------+---------+---------+-
                                       -0.050     0.000     0.050     0.100


S6 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S7  -0.03733   0.00383  0.04500            (-------*-------)
S8  -0.07783  -0.03667  0.00450    (--------*-------)
                                  --------+---------+---------+---------+-
                                       -0.050     0.000     0.050     0.100


S7 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S8  -0.08167  -0.04050  0.00067    (-------*-------)
                                  --------+---------+---------+---------+-
                                       -0.050     0.000     0.050     0.100
```

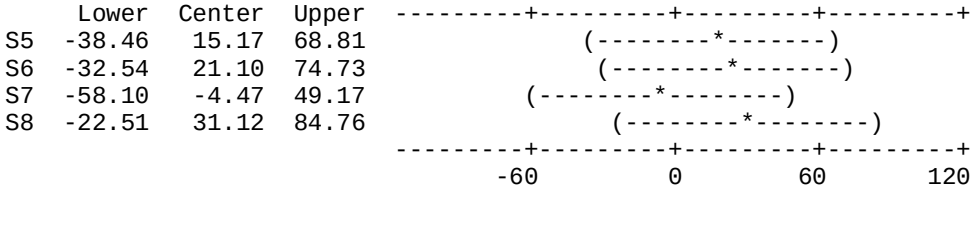
One-way ANOVA: Modulus

```
Source  DF     SS    MS     F      P
Factor   7  16752  2393  2.83  0.017
Error   40  33792   845
Total   47  50544

S = 29.07   R-Sq = 33.14%   R-Sq(adj) = 21.44%


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N   Mean  StDev  -------+---------+---------+---------+--
S1     6  80.84  24.33                (-------*-------)
S2     6  92.71  35.13                    (-------*-------)
S3     6  58.33  20.24         (-------*-------)
S4     6  38.27  12.85    (-------*-------)
S5     6  53.44  55.34         (-------*-------)
S6     6  59.37  27.61           (-------*-------)
S7     6  33.80   8.91  (-------*-------)
S8     6  69.39  21.30             (-------*-------)
                      -------+---------+---------+---------+--
                            30        60        90       120

Pooled StDev = 29.07


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.73%


S1 subtracted from:

      Lower  Center  Upper  ---------+---------+---------+---------+
S2   -41.76   11.87  65.51              (--------*--------)
S3   -76.14  -22.51  31.13        (--------*--------)
S4   -96.20  -42.56  11.07     (--------*--------)
S5   -81.03  -27.39  26.24       (--------*--------)
S6   -75.10  -21.46  32.17         (--------*--------)
S7  -100.66  -47.03   6.60    (--------*--------)
S8   -65.07  -11.44  42.19          (--------*--------)
                           ---------+---------+---------+---------+
                                  -60         0        60       120


S2 subtracted from:

      Lower  Center  Upper  ---------+---------+---------+---------+
S3   -88.01  -34.38  19.25      (--------*--------)
S4  -108.07  -54.44  -0.80   (--------*--------)
S5   -92.90  -39.27  14.37      (--------*--------)
S6   -86.97  -33.34  20.30       (--------*--------)
S7  -112.54  -58.91  -5.27  (--------*--------)
S8   -76.95  -23.31  30.32        (--------*--------)
                           ---------+---------+---------+---------+
                                  -60         0        60       120
```

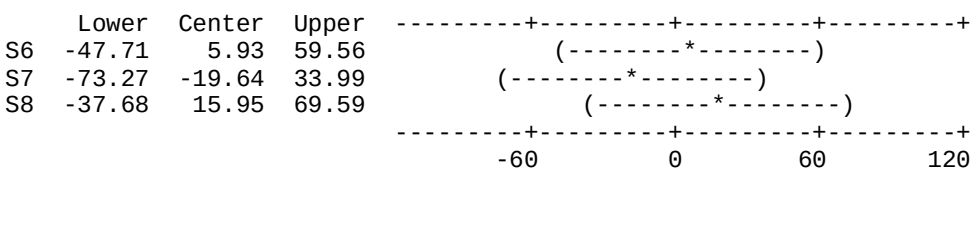
S3 subtracted from:



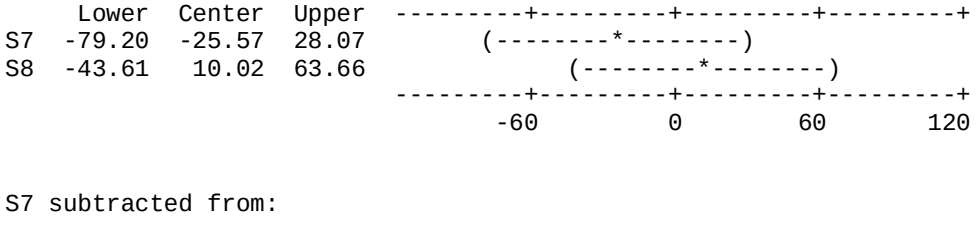
S4 subtracted from:



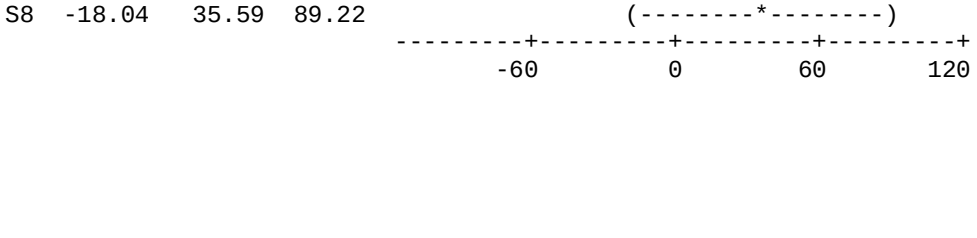
S5 subtracted from:



S6 subtracted from:

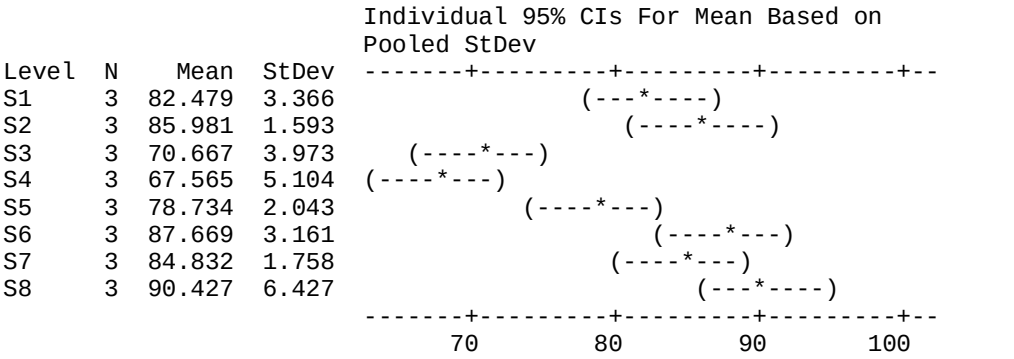


S7 subtracted from:



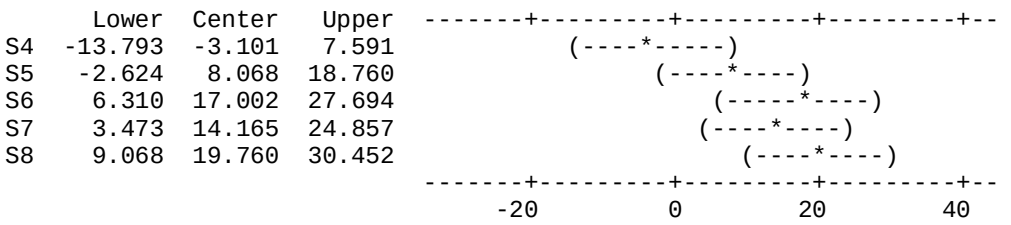
One-way ANOVA: Porosity

```
Source  DF      SS     MS      F      P
Factor   7  1402.2  200.3  14.02  0.000
Error   16   228.5   14.3
Total   23  1630.7

S = 3.779   R-Sq = 85.99%   R-Sq(adj) = 79.85%
```

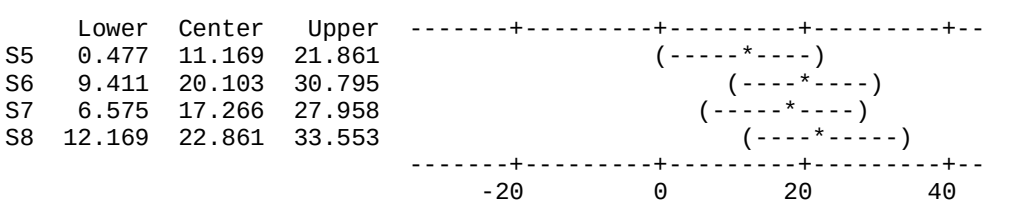



```
Pooled StDev = 3.779


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.68%


S1 subtracted from:

      Lower   Center   Upper
S2   -7.190    3.501  14.193
S3  -22.505  -11.813  -1.121
S4  -25.606  -14.914  -4.222
S5  -14.437   -3.745   6.947
S6   -5.503    5.189  15.881
S7   -8.339    2.352  13.044
S8   -2.745    7.947  18.639
                      -20         0        20        40


S2 subtracted from:

      Lower   Center   Upper
S3  -26.006  -15.314  -4.622
S4  -29.107  -18.416  -7.724
S5  -17.938   -7.247   3.445
S6   -9.004    1.688  12.380
S7  -11.841   -1.149   9.543
S8   -6.246    4.446  15.138
                      -20         0        20        40
```

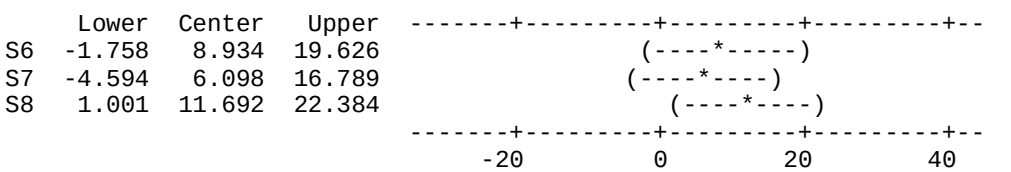
S3 subtracted from:



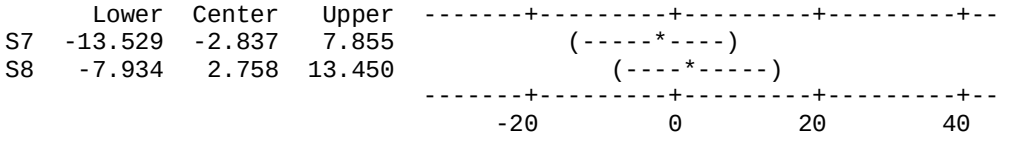
S4 subtracted from:



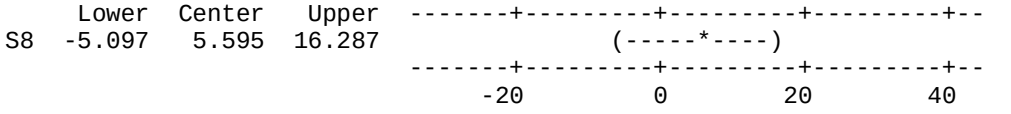
S5 subtracted from:



S6 subtracted from:



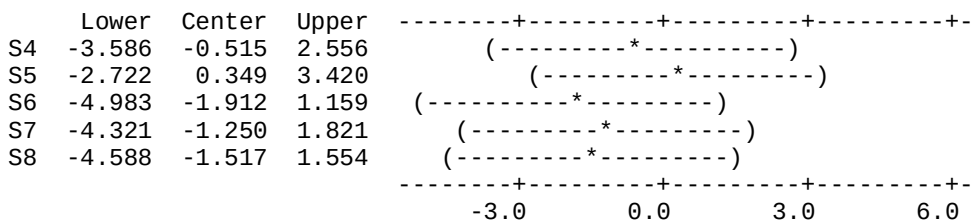
S7 subtracted from:



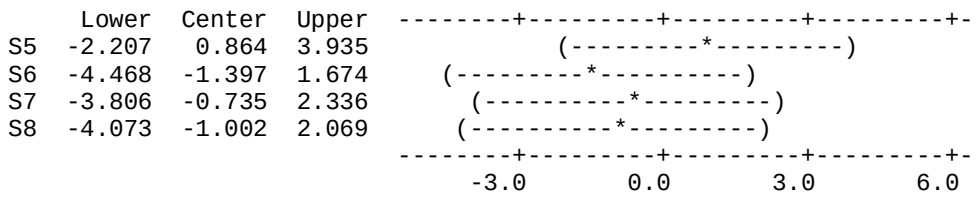
One-way ANOVA: Permeability

```
Source  DF     SS    MS     F      P
Factor   7  12.81  1.83  1.55  0.220
Error   16  18.86  1.18
Total   23  31.67

S = 1.086   R-Sq = 40.46%   R-Sq(adj) = 14.41%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N   Mean  StDev  --------+---------+---------+---------+-
S1     3  1.713  1.421            (-------*--------)
S2     3  1.690  1.343           (--------*--------)
S3     3  2.079  0.636              (--------*--------)
S4     3  1.564  1.115           (-------*--------)
S5     3  2.428  1.229                (--------*--------)
S6     3  0.167  0.014  (--------*--------)
S7     3  0.829  1.283      (--------*-------)
S8     3  0.562  0.896    (--------*--------)
                        --------+---------+---------+---------+-
                              0.0       1.5       3.0       4.5

Pooled StDev = 1.086


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.68%


S1 subtracted from:

     Lower  Center  Upper  --------+---------+---------+---------+-
S2  -3.095  -0.023  3.048          (---------*---------)
S3  -2.705   0.366  3.437           (---------*---------)
S4  -3.220  -0.149  2.922         (----------*---------)
S5  -2.356   0.715  3.786            (---------*----------)
S6  -4.617  -1.546  1.525     (---------*---------)
S7  -3.955  -0.884  2.187       (---------*---------)
S8  -4.222  -1.151  1.920     (---------*---------)
                           --------+---------+---------+---------+-
                                -3.0       0.0       3.0       6.0


S2 subtracted from:

     Lower  Center  Upper  --------+---------+---------+---------+-
S3  -2.682   0.389  3.461          (---------*----------)
S4  -3.197  -0.126  2.946         (----------*---------)
S5  -2.333   0.738  3.810            (---------*----------)
S6  -4.594  -1.523  1.549     (---------*---------)
S7  -3.932  -0.861  2.211       (---------*---------)
S8  -4.199  -1.128  1.944     (---------*---------)
                           --------+---------+---------+---------+-
                                -3.0       0.0       3.0       6.0
```

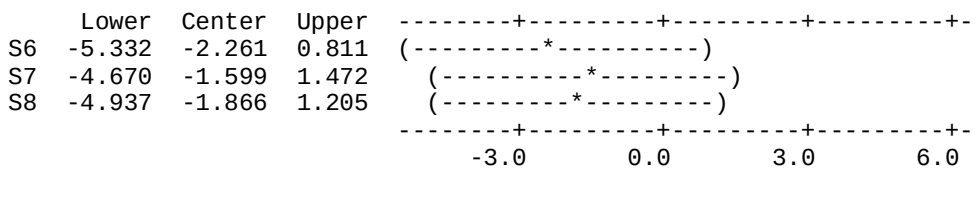
S3 subtracted from:



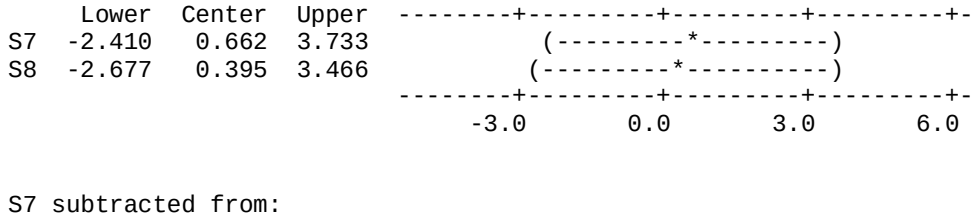
S4 subtracted from:



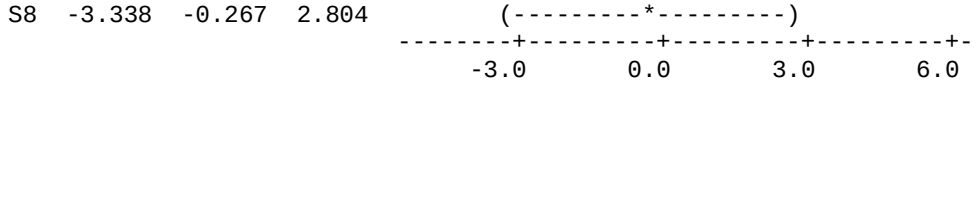
S5 subtracted from:



S6 subtracted from:



S7 subtracted from:



One-way ANOVA: Pore size

```
Source  DF      SS      MS     F      P
Factor   7  0.9393  0.1342  1.99  0.120
Error   16  1.0787  0.0674
Total   23  2.0180

S = 0.2596   R-Sq = 46.55%   R-Sq(adj) = 23.16%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean   StDev  ---+---------+---------+---------+------
S1     3  0.6106  0.3277            (--------*---------)
S2     3  0.6299  0.2496             (--------*--------)
S3     3  0.7286  0.1124                (--------*--------)
S4     3  0.5942  0.2810            (--------*--------)
S5     3  0.7729  0.2207                 (--------*--------)
S6     3  0.2082  0.0088  (--------*--------)
S7     3  0.3583  0.3605     (--------*--------)
S8     3  0.2821  0.3151   (--------*--------)
                         ---+---------+---------+---------+------
                          0.00      0.35      0.70      1.05

Pooled StDev = 0.2596


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.68%


S1 subtracted from:

      Lower   Center   Upper  ---------+---------+---------+---------+
S2  -0.7152   0.0193  0.7539           (---------*----------)
S3  -0.6165   0.1180  0.8526             (----------*---------)
S4  -0.7509  -0.0164  0.7182          (----------*---------)
S5  -0.5723   0.1623  0.8968             (---------*----------)
S6  -1.1369  -0.4024  0.3322     (---------*----------)
S7  -0.9868  -0.2523  0.4823       (---------*----------)
S8  -1.0630  -0.3284  0.4061      (---------*----------)
                         ---------+---------+---------+---------+
                               -0.70      0.00      0.70      1.40


S2 subtracted from:

      Lower   Center   Upper  ---------+---------+---------+---------+
S3  -0.6358   0.0987  0.8333           (---------*----------)
S4  -0.7702  -0.0357  0.6989          (----------*----------)
S5  -0.5916   0.1430  0.8775             (---------*----------)
S6  -1.1562  -0.4217  0.3129    (----------*---------)
S7  -1.0061  -0.2716  0.4630       (---------*----------)
S8  -1.0823  -0.3478  0.3868      (---------*----------)
                         ---------+---------+---------+---------+
                               -0.70      0.00      0.70      1.40
```

```
S3 subtracted from:

      Lower   Center   Upper  ---------+---------+---------+---------+
S4  -0.8690  -0.1344  0.6001         (---------*---------)
S5  -0.6903   0.0443  0.7788           (----------*---------)
S6  -1.2550  -0.5204  0.2141   (----------*---------)
S7  -1.1048  -0.3703  0.3643     (----------*---------)
S8  -1.1810  -0.4465  0.2881    (----------*---------)
                              ---------+---------+---------+---------+
                                    -0.70      0.00      0.70      1.40


S4 subtracted from:

      Lower   Center   Upper  ---------+---------+---------+---------+
S5  -0.5559   0.1787  0.9132             (----------*---------)
S6  -1.1205  -0.3860  0.3486     (---------*----------)
S7  -0.9704  -0.2359  0.4987       (----------*---------)
S8  -1.0466  -0.3121  0.4225      (----------*---------)
                              ---------+---------+---------+---------+
                                    -0.70      0.00      0.70      1.40


S5 subtracted from:

      Lower   Center   Upper  ---------+---------+---------+---------+
S6  -1.2992  -0.5647  0.1699  (----------*---------)
S7  -1.1491  -0.4145  0.3200     (---------*----------)
S8  -1.2253  -0.4907  0.2438   (----------*---------)
                              ---------+---------+---------+---------+
                                    -0.70      0.00      0.70      1.40


S6 subtracted from:

      Lower  Center   Upper  ---------+---------+---------+---------+
S7  -0.5844  0.1501  0.8847             (---------*----------)
S8  -0.6606  0.0739  0.8085           (---------*----------)
                             ---------+---------+---------+---------+
                                   -0.70      0.00      0.70      1.40


S7 subtracted from:

      Lower   Center   Upper  ---------+---------+---------+---------+
S8  -0.8107  -0.0762  0.6584         (----------*---------)
                              ---------+---------+---------+---------+
                                    -0.70      0.00      0.70      1.40
```

One-way ANOVA: Swelling studies (data points at 48 h)

```
Source  DF      SS     MS     F      P
Factor   7  264049  37721  1.06  0.429
Error   16  567362  35460
Total   23  831410

S = 188.3   R-Sq = 31.76%   R-Sq(adj) = 1.90%


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N   Mean  StDev   -+---------+---------+---------+--------
S1     3  595.4  144.9            (-----------*----------)
S2     3  409.6   75.6   (----------*-----------)
S3     3  650.1  233.7              (-----------*----------)
S4     3  735.1  330.3                  (-----------*----------)
S5     3  498.5  154.5       (-----------*----------)
S6     3  717.0  168.1                 (-----------*----------)
S7     3  527.3  188.1         (----------*-----------)
S8     3  538.6   76.0         (-----------*----------)
                       -+---------+---------+---------+--------
                      200       400       600       800

Pooled StDev = 188.3


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.68%


S1 subtracted from:

     Lower  Center  Upper  -------+---------+---------+---------+--
S2  -718.5  -185.8  346.9     (---------*----------)
S3  -478.1    54.7  587.4         (----------*----------)
S4  -393.1   139.7  672.4           (----------*---------)
S5  -629.6   -96.9  435.8      (----------*----------)
S6  -411.1   121.6  654.4           (---------*----------)
S7  -600.9   -68.1  464.6       (----------*---------)
S8  -589.5   -56.8  476.0       (----------*----------)
                          -------+---------+---------+---------+--
                              -500         0       500      1000


S2 subtracted from:

     Lower  Center  Upper  -------+---------+---------+---------+--
S3  -292.2   240.5  773.2             (----------*---------)
S4  -207.3   325.5  858.2               (----------*---------)
S5  -443.8    88.9  621.7          (----------*---------)
S6  -225.3   307.4  840.2             (----------*----------)
S7  -415.1   117.7  650.4           (---------*----------)
S8  -403.7   129.0  661.8           (----------*---------)
                          -------+---------+---------+---------+--
                              -500         0       500      1000
```

```
S3 subtracted from:

     Lower  Center  Upper  -------+---------+---------+---------+--
S4  -447.7    85.0  617.7         (----------*---------)
S5  -684.3  -151.6  381.2    (----------*----------)
S6  -465.8    67.0  599.7         (---------*----------)
S7  -655.5  -122.8  409.9      (----------*---------)
S8  -644.2  -111.4  421.3      (----------*---------)
                           -------+---------+---------+---------+--
                               -500         0       500      1000


S4 subtracted from:

     Lower  Center  Upper  -------+---------+---------+---------+--
S5  -769.3  -236.5  296.2   (---------*----------)
S6  -550.7   -18.0  514.7       (----------*---------)
S7  -740.5  -207.8  324.9    (----------*---------)
S8  -729.1  -196.4  336.3    (----------*----------)
                           -------+---------+---------+---------+--
                               -500         0       500      1000


S5 subtracted from:

     Lower  Center  Upper  -------+---------+---------+---------+--
S6  -314.2   218.5  751.2            (---------*----------)
S7  -504.0    28.7  561.5        (----------*---------)
S8  -492.6    40.1  572.9        (----------*---------)
                           -------+---------+---------+---------+--
                               -500         0       500      1000


S6 subtracted from:

     Lower  Center  Upper  -------+---------+---------+---------+--
S7  -722.5  -189.8  342.9    (---------*----------)
S8  -711.1  -178.4  354.3    (---------*----------)
                           -------+---------+---------+---------+--
                               -500         0       500      1000


S7 subtracted from:

     Lower  Center  Upper  -------+---------+---------+---------+--
S8  -521.3    11.4  544.1        (---------*----------)
                           -------+---------+---------+---------+--
                               -500         0       500      1000
```

Degradation studies for scaffold S1

One-way ANOVA: cell conditioned medium at 37°C

```
Source  DF      SS     MS     F      P
Factor   2   765.3  382.7  9.04  0.015
Error    6   254.0   42.3
Total    8  1019.3

S = 6.506   R-Sq = 75.09%   R-Sq(adj) = 66.78%


                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level          N    Mean  StDev  ---------+---------+---------+---------+
CCM @ 37C  6h  3   80.44  11.27  (---------*---------)
CCM @ 37C 12h  3  100.00   0.00                     (---------*---------)
CCM @ 37C 24h  3  100.00   0.00                     (---------*---------)
                                ---------+---------+---------+---------+
                                        80        90       100       110

Pooled StDev = 6.51


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 97.80%


CCM @ 37C  6h subtracted from:

               Lower  Center   Upper   -+---------+---------+---------+--------
CCM @ 37C 12h  3.260  19.562  35.864               (----------*----------)
CCM @ 37C 24h  3.260  19.562  35.864               (----------*----------)
                                     -+---------+---------+---------+--------
                                    -15         0        15        30


CCM @ 37C 12h subtracted from:

                 Lower  Center   Upper
CCM @ 37C 24h  -16.302   0.000  16.302

                -+---------+---------+---------+--------
CCM @ 37C 24h   (----------*----------)
                -+---------+---------+---------+--------
               -15         0        15        30
```

One-way ANOVA: DMEM at 37°C

```
Source  DF      SS     MS      F      P
Factor   2  1372.1  686.1  17.12  0.003
Error    6   240.4   40.1
Total    8  1612.5

S = 6.330   R-Sq = 85.09%   R-Sq(adj) = 80.12%


                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level            N    Mean  StDev  ---------+---------+---------+---------+
DMEM @ 37C  6h   3   70.06   5.48  (------*-------)
DMEM @ 37C  12h  3   81.31   9.49           (-------*------)
DMEM @ 37C  24h  3  100.00   0.00                          (------*-------)
                                    ---------+---------+---------+---------+
                                            72        84        96       108

Pooled StDev = 6.33


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 97.80%


DMEM @ 37C  6h subtracted from:

                 Lower  Center   Upper
DMEM @ 37C  12h  -4.612  11.249  27.109
DMEM @ 37C  24h  14.078  29.938  45.799

                 ----+---------+---------+---------+-----
DMEM @ 37C  12h             (-----*------)
DMEM @ 37C  24h                     (-----*-----)
                 ----+---------+---------+---------+-----
                   -25         0        25        50


DMEM @ 37C  12h subtracted from:

                 Lower  Center   Upper
DMEM @ 37C  24h  2.829  18.689  34.550

                 ----+---------+---------+---------+-----
DMEM @ 37C  24h               (-----*------)
                 ----+---------+---------+---------+-----
                   -25         0        25        50
```

One-way ANOVA: DMEM at room temperature

```
Source  DF     SS    MS     F      P
Factor   2   17.0   8.5  0.35  0.715
Error    6  143.6  23.9
Total    8  160.6

S = 4.892   R-Sq = 10.56%   R-Sq(adj) = 0.00%


                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level              N   Mean  StDev   -+---------+---------+---------+--------
DMEM room tmp 6h   3  8.718  7.543        (------------*-------------)
DMEM room tmp 12h  3  9.848  1.488          (-------------*-------------)
DMEM room tmp 24h  3  6.541  3.562   (-------------*-------------)
                                     -+---------+---------+---------+--------
                                    0.0       5.0      10.0      15.0

Pooled StDev = 4.892


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 97.80%


DMEM room tmp 6h subtracted from:

                     Lower  Center   Upper
DMEM room tmp 12h  -11.128   1.131  13.389
DMEM room tmp 24h  -14.435  -2.177  10.082

                   ---------+---------+---------+---------+
DMEM room tmp 12h       (--------------*---------------)
DMEM room tmp 24h   (--------------*---------------)
                   ---------+---------+---------+---------+
                         -8.0       0.0       8.0      16.0


DMEM room tmp 12h subtracted from:

                     Lower  Center  Upper
DMEM room tmp 24h  -15.566  -3.307  8.951

                   ---------+---------+---------+---------+
DMEM room tmp 24h  (--------------*--------------)
                   ---------+---------+---------+---------+
                         -8.0       0.0       8.0      16.0
```

One-way ANOVA: SWF at 37°C

```
Source  DF      SS      MS      F      P
Factor   2  6737.5  3368.7  49.58  0.000
Error    6   407.6    67.9
Total    8  7145.1

S = 8.243   R-Sq = 94.29%   R-Sq(adj) = 92.39%


                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level          N    Mean  StDev  -+---------+---------+---------+--------
SWF @ 37C 6h   3   33.48  10.10  (---*----)
SWF @ 37C 12h  3   73.81  10.09                (----*---)
SWF @ 37C 24h  3  100.00   0.00                          (----*----)
                                -+---------+---------+---------+--------
                                25        50        75       100

Pooled StDev = 8.24


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 97.80%


SWF @ 37C 6h subtracted from:

                Lower  Center   Upper  ---+---------+---------+---------+------
SWF @ 37C 12h  19.680  40.334  60.987                   (-----*----)
SWF @ 37C 24h  45.867  66.520  87.174                         (-----*-----)
                                     ---+---------+---------+---------+------
                                      -35         0        35        70


SWF @ 37C 12h subtracted from:

               Lower  Center   Upper  ---+---------+---------+---------+------
SWF @ 37C 24h  5.533  26.186  46.840               (----*-----)
                                     ---+---------+---------+---------+------
                                      -35         0        35        70
```

One-way ANOVA: Data at 6h from all media

```
Source  DF       SS      MS      F      P
Factor   3   9878.3  3292.8  41.70  0.000
Error    8    631.8    79.0
Total   11  10510.1

S = 8.887   R-Sq = 93.99%   R-Sq(adj) = 91.73%


                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level             N    Mean   StDev  -+---------+---------+---------+--------
CCM @ 37C  6h     3  80.438  11.268                              (----*----)
DMEM @ 37C  6h    3  70.062   5.484                         (----*----)
DMEM room tmp 6h  3   8.718   7.543  (---*----)
SWF @ 37C 6h      3  33.480  10.096            (---*----)
                                    -+---------+---------+---------+--------
                                     0        25        50        75

Pooled StDev = 8.887


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.74%


CCM @ 37C  6h subtracted from:

                  Lower  Center   Upper
DMEM @ 37C  6h    -33.62  -10.38   12.87
DMEM room tmp 6h  -94.96  -71.72  -48.48
SWF @ 37C 6h      -70.20  -46.96  -23.72

                  ---------+---------+---------+---------+
DMEM @ 37C  6h               (----*----)
DMEM room tmp 6h  (----*---)
SWF @ 37C 6h           (----*---)
                  ---------+---------+---------+---------+
                         -50         0        50       100

DMEM @ 37C  6h subtracted from:

                  Lower  Center   Upper
DMEM room tmp 6h  -84.59  -61.34  -38.10
SWF @ 37C 6h      -59.82  -36.58  -13.34

                  ---------+---------+---------+---------+
DMEM room tmp 6h    (----*---)
SWF @ 37C 6h             (----*---)
                  ---------+---------+---------+---------+
                         -50         0        50       100

DMEM room tmp 6h subtracted from:

              Lower  Center  Upper  ---------+---------+---------+---------+
SWF @ 37C 6h   1.52   24.76  48.00                     (----*----)
                                   ---------+---------+---------+---------+
                                          -50         0        50       100
```

One-way ANOVA: Data at 12h from all media

```
Source  DF       SS      MS      F      P
Factor   3  13812.7  4604.2  94.82  0.000
Error    8    388.4    48.6
Total   11  14201.1

S = 6.968   R-Sq = 97.26%   R-Sq(adj) = 96.24%


                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level              N    Mean  StDev  +---------+---------+---------+---------
CCM @ 37C 12h      3  100.00   0.00                                (--*--)
DMEM @ 37C  12h    3   81.31   9.49                           (--*--)
DMEM room tmp 12h  3    9.85   1.49  (--*--)
SWF @ 37C 12h      3   73.81  10.09                      (--*--)
                                    +---------+---------+---------+---------
                                    0        30        60        90

Pooled StDev = 6.97


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.74%


CCM @ 37C 12h subtracted from:

                    Lower  Center   Upper
DMEM @ 37C  12h     -36.91  -18.69   -0.46
DMEM room tmp 12h  -108.38  -90.15  -71.93
SWF @ 37C 12h       -44.41  -26.19   -7.96

                    --+---------+---------+---------+-------
DMEM @ 37C  12h                 (--*---)
DMEM room tmp 12h   (---*---)
SWF @ 37C 12h                  (---*--)
                    --+---------+---------+---------+-------
                   -100       -50         0        50

DMEM @ 37C  12h subtracted from:

                    Lower  Center   Upper
DMEM room tmp 12h  -89.69  -71.46  -53.24
SWF @ 37C 12h      -25.72   -7.50   10.73

                    --+---------+---------+---------+-------
DMEM room tmp 12h       (---*--)
SWF @ 37C 12h                    (---*--)
                    --+---------+---------+---------+-------
                   -100       -50         0        50

DMEM room tmp 12h subtracted from:

               Lower  Center  Upper   --+---------+---------+---------+-------
SWF @ 37C 12h  45.74   63.97  82.19                                  (---*--)
                                        --+---------+---------+---------+-------
                                       -100       -50         0        50
```

One-way ANOVA: Data at 24h from all media

```
Source  DF        SS       MS        F      P
Factor   3  19652.72  6550.91  2065.00  0.000
Error    8     25.38     3.17
Total   11  19678.10

S = 1.781   R-Sq = 99.87%   R-Sq(adj) = 99.82%


                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level              N    Mean  StDev  --------+---------+---------+---------+-
CCM @ 37C 24h      3  100.00   0.00                                       (*)
DMEM @ 37C  24h    3  100.00   0.00                                       (*)
DMEM room tmp 24h  3    6.54   3.56  (*)
SWF @ 37C 24h      3  100.00   0.00                                       (*)
                                    --------+---------+---------+---------+-
                                           25        50        75       100
Pooled StDev = 1.78

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.74%

CCM @ 37C 24h subtracted from:

                    Lower  Center   Upper
DMEM @ 37C  24h     -4.66    0.00    4.66
DMEM room tmp 24h  -98.12  -93.46  -88.80
SWF @ 37C 24h       -4.66    0.00    4.66

                      +---------+---------+---------+---------
DMEM @ 37C  24h                         (*)
DMEM room tmp 24h     (*)
SWF @ 37C 24h                           (*)
                      +---------+---------+---------+---------
                    -100       -50         0        50

DMEM @ 37C  24h subtracted from:

                    Lower  Center   Upper
DMEM room tmp 24h  -98.12  -93.46  -88.80
SWF @ 37C 24h       -4.66    0.00    4.66

                      +---------+---------+---------+---------
DMEM room tmp 24h     (*)
SWF @ 37C 24h                           (*)
                      +---------+---------+---------+---------
                    -100       -50         0        50

DMEM room tmp 24h subtracted from:

                 Lower  Center  Upper
SWF @ 37C 24h    88.80   93.46  98.12

                    +---------+---------+---------+---------
SWF @ 37C 24h                                       (*)
                    +---------+---------+---------+---------
                  -100       -50         0        50
```

Degradation studies for scaffold S5

One-way ANOVA: Cell conditioned medium at 37°C

```
Source  DF       SS      MS       F      P
Factor   2  1092.70  546.35  166.19  0.000
Error    6    19.73    3.29
Total    8  1112.42

S = 1.813   R-Sq = 98.23%   R-Sq(adj) = 97.64%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level    N    Mean  StDev  -------+---------+---------+---------+--
CCM 6h   3   76.63   3.14  (--*--)
CCM 12h  3  100.00   0.00                              (--*--)
CCM 24h  3  100.00   0.00                              (--*--)
                          -------+---------+---------+---------+--
                              80.0      88.0      96.0     104.0

Pooled StDev = 1.81


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 97.80%


CCM 6h subtracted from:

          Lower  Center   Upper  -----+---------+---------+---------+----
CCM 12h  18.831  23.374  27.917                          (---*----)
CCM 24h  18.831  23.374  27.917                          (---*----)
                                -----+---------+---------+---------+----
                                     0        10        20        30


CCM 12h subtracted from:

          Lower  Center  Upper  -----+---------+---------+---------+----
CCM 24h  -4.543   0.000  4.543  (----*----)
                                -----+---------+---------+---------+----
                                     0        10        20        30
```

One-way ANOVA: DMEM at 37°C

```
Source  DF    SS    MS      F      P
Factor   2  7014  3507  13.76  0.006
Error    6  1529   255
Total    8  8543

S = 15.96   R-Sq = 82.10%   R-Sq(adj) = 76.14%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level            N    Mean  StDev  -----+---------+---------+---------+----
DMEM @ 37C  6h   3   37.52   9.95  (-------*------)
DMEM @ 37C  12h  3   44.69  25.80    (-------*------)
DMEM @ 37C  24h  3  100.00   0.00                      (------*-------)
                                  -----+---------+---------+---------+----
                                      30        60        90       120

Pooled StDev = 15.96


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 97.80%


DMEM @ 37C  6h subtracted from:

                 Lower  Center   Upper
DMEM @ 37C  12h  -32.83    7.17   47.17
DMEM @ 37C  24h   22.48   62.48  102.48

                 ---------+---------+---------+---------+
DMEM @ 37C  12h             (-------*-------)
DMEM @ 37C  24h                       (-------*-------)
                 ---------+---------+---------+---------+
                       -50         0        50       100


DMEM @ 37C  12h subtracted from:

                 Lower  Center  Upper  ---------+---------+---------+---------+
DMEM @ 37C  24h  15.31   55.31  95.31                      (-------*-------)
                                       ---------+---------+---------+---------+
                                              -50         0        50       100
```

One-way ANOVA: DMEM at room temperature

```
Source  DF     SS    MS     F      P
Factor   2   88.0  44.0  0.72  0.524
Error    6  366.2  61.0
Total    8  454.2

S = 7.812   R-Sq = 19.38%   R-Sq(adj) = 0.00%


                                      Individual 95% CIs For Mean Based on
                                      Pooled StDev
Level              N    Mean   StDev  -------+---------+---------+---------+--
DMEM room tmp 6h   3  13.705  10.047  (-------------*-------------)
DMEM room tmp 12h  3  16.939   9.025      (-------------*-------------)
DMEM room tmp 24h  3  21.337   0.829            (-------------*-------------)
                                      -------+---------+---------+---------+--
                                           8.0      16.0      24.0      32.0

Pooled StDev = 7.812


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 97.80%


DMEM room tmp 6h subtracted from:

                     Lower  Center   Upper
DMEM room tmp 12h  -16.342   3.234  22.809
DMEM room tmp 24h  -11.943   7.632  27.207

                   ------+---------+---------+---------+---
DMEM room tmp 12h       (------------*------------)
DMEM room tmp 24h          (------------*------------)
                   ------+---------+---------+---------+---
                       -15         0        15        30


DMEM room tmp 12h subtracted from:

                     Lower  Center   Upper
DMEM room tmp 24h  -15.177   4.398  23.974

                   ------+---------+---------+---------+---
DMEM room tmp 24h        (------------*------------)
                   ------+---------+---------+---------+---
                       -15         0        15        30
```

One-way ANOVA: SWF at 37°C

```
Source  DF      SS      MS       F      P
Factor   2  7509.6  3754.8  187.64  0.000
Error    6   120.1    20.0
Total    8  7629.7

S = 4.473   R-Sq = 98.43%   R-Sq(adj) = 97.90%


                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level          N    Mean  StDev   +---------+---------+---------+---------
SWF @ 37C 6h   3   30.35   4.69   (-*--)
SWF @ 37C 12h  3   54.39   6.17            (--*-)
SWF @ 37C 24h  3  100.00   0.00                              (--*--)
                                  +---------+---------+---------+---------
                                  25        50        75       100

Pooled StDev = 4.47


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 97.80%


SWF @ 37C 6h subtracted from:

                Lower  Center   Upper  ------+---------+---------+---------+---
SWF @ 37C 12h  12.831  24.040  35.249                       (--*--)
SWF @ 37C 24h  58.443  69.651  80.860                                 (--*--)
                                       ------+---------+---------+---------+---
                                           -35         0        35        70


SWF @ 37C 12h subtracted from:

                Lower  Center   Upper  ------+---------+---------+---------+---
SWF @ 37C 24h  34.403  45.611  56.820                            (--*--)
                                       ------+---------+---------+---------+---
                                           -35         0        35        70
```

One-way ANOVA: Data at 6h from all media

```
Source  DF      SS      MS      F      P
Factor   3  6394.0  2131.3  36.77  0.000
Error    8   463.8    58.0
Total   11  6857.8

S = 7.614   R-Sq = 93.24%   R-Sq(adj) = 90.70%


                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level             N    Mean   StDev  ---------+---------+---------+---------+
CCM 6h            3  76.626   3.140                           (---*---)
DMEM @ 37C  6h    3  37.523   9.953            (---*---)
DMEM room tmp 6h  3  13.705  10.047  (---*----)
SWF @ 37C 6h      3  30.349   4.692         (---*---)
                                    ---------+---------+---------+---------+
                                           25        50        75       100

Pooled StDev = 7.614


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.74%

CCM 6h subtracted from:

                    Lower   Center    Upper
DMEM @ 37C  6h    -59.016  -39.102  -19.189
DMEM room tmp 6h  -82.834  -62.921  -43.007
SWF @ 37C 6h      -66.190  -46.277  -26.364

                  ----+---------+---------+---------+-----
DMEM @ 37C  6h           (-----*-----)
DMEM room tmp 6h  (-----*-----)
SWF @ 37C 6h           (-----*----)
                  ----+---------+---------+---------+-----
                    -70       -35         0        35

DMEM @ 37C  6h subtracted from:

                    Lower   Center   Upper
DMEM room tmp 6h  -43.732  -23.818  -3.905
SWF @ 37C 6h      -27.088   -7.175  12.739

                  ----+---------+---------+---------+-----
DMEM room tmp 6h             (----*-----)
SWF @ 37C 6h                    (-----*-----)
                  ----+---------+---------+---------+-----
                    -70       -35         0        35


DMEM room tmp 6h subtracted from:

                Lower  Center   Upper  ----+---------+---------+---------+-----
SWF @ 37C 6h  -3.270  16.643  36.557                          (-----*----)
                                       ----+---------+---------+---------+-----
                                         -70       -35         0        35
```

One-way ANOVA: Data at 12 h from all media

```
Source  DF     SS    MS      F      P
Factor   3  10729  3576  18.23  0.001
Error    8   1570   196
Total   11  12299

S = 14.01   R-Sq = 87.24%   R-Sq(adj) = 82.45%


                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level              N    Mean  StDev  +---------+---------+---------+---------
CCM 12h            3  100.00   0.00                        (-----*----)
DMEM @ 37C  12h    3   44.69  25.80         (-----*----)
DMEM room tmp 12h  3   16.94   9.03  (----*----)
SWF @ 37C 12h      3   54.39   6.17            (-----*----)
                                    +---------+---------+---------+---------
                                    0        35        70       105
Pooled StDev = 14.01

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.74%

CCM 12h subtracted from:

                     Lower  Center   Upper
DMEM @ 37C  12h     -91.94  -55.31  -18.67
DMEM room tmp 12h  -119.70  -83.06  -46.43
SWF @ 37C 12h       -82.25  -45.61   -8.98

                   ----+---------+---------+---------+-----
DMEM @ 37C  12h          (------*------)
DMEM room tmp 12h  (------*-------)
SWF @ 37C 12h              (------*------)
                   ----+---------+---------+---------+-----
                    -100       -50         0        50

DMEM @ 37C  12h subtracted from:

                    Lower  Center  Upper
DMEM room tmp 12h  -64.39  -27.75   8.88
SWF @ 37C 12h      -26.94    9.70  46.33

                   ----+---------+---------+---------+-----
DMEM room tmp 12h             (------*-------)
SWF @ 37C 12h                        (------*------)
                   ----+---------+---------+---------+-----
                    -100       -50         0        50


DMEM room tmp 12h subtracted from:

                Lower  Center  Upper  ----+---------+---------+---------+-----
SWF @ 37C 12h    0.81   37.45  74.09                          (------*-------)
                                       ----+---------+---------+---------+-----
                                        -100       -50         0        50
```

One-way ANOVA: Data at 24h from all media

```
Source  DF        SS       MS         F      P
Factor   3  13922.63  4640.88  27025.15  0.000
Error    8      1.37     0.17
Total   11  13924.01

S = 0.4144   R-Sq = 99.99%   R-Sq(adj) = 99.99%



Level              N     Mean  StDev
CCM 24h            3  100.000  0.000
DMEM @ 37C  24h    3  100.000  0.000
DMEM room tmp 24h  3   21.337  0.829
SWF @ 37C 24h      3  100.000  0.000

                   Individual 95% CIs For Mean Based on Pooled StDev
Level               +---------+---------+---------+---------
CCM 24h                                              *
DMEM @ 37C  24h                                      *
DMEM room tmp 24h   (*
SWF @ 37C 24h                                        *
                    +---------+---------+---------+---------
                   20        40        60        80

Pooled StDev = 0.414


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.74%


CCM 24h subtracted from:

                     Lower   Center    Upper
DMEM @ 37C  24h     -1.084    0.000    1.084
DMEM room tmp 24h  -79.747  -78.663  -77.579
SWF @ 37C 24h       -1.084    0.000    1.084

                     +---------+---------+---------+---------
DMEM @ 37C  24h                          *
DMEM room tmp 24h    *)
SWF @ 37C 24h                            *
                     +---------+---------+---------+---------
                   -80       -40         0        40


DMEM @ 37C  24h subtracted from:

                     Lower   Center    Upper
DMEM room tmp 24h  -79.747  -78.663  -77.579
SWF @ 37C 24h       -1.084    0.000    1.084

                     +---------+---------+---------+---------
DMEM room tmp 24h    *)
SWF @ 37C 24h                            *
                     +---------+---------+---------+---------
                   -80       -40         0        40
```

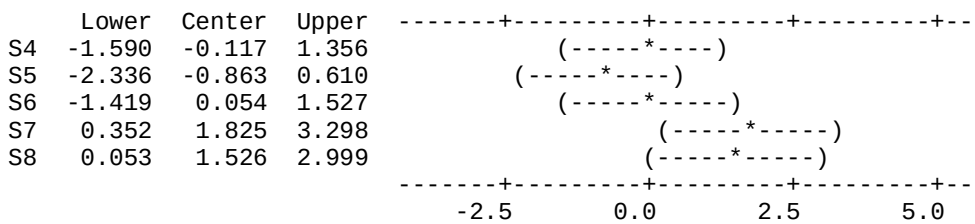
```
DMEM room tmp 24h subtracted from:

                 Lower  Center   Upper
SWF @ 37C 24h  77.579  78.663  79.747

                  +---------+---------+---------+---------
SWF @ 37C 24h                                         (*
                  +---------+---------+---------+---------
                -80       -40         0        40
```

Appendix B (Data for the non-crosslinked scaffolds)

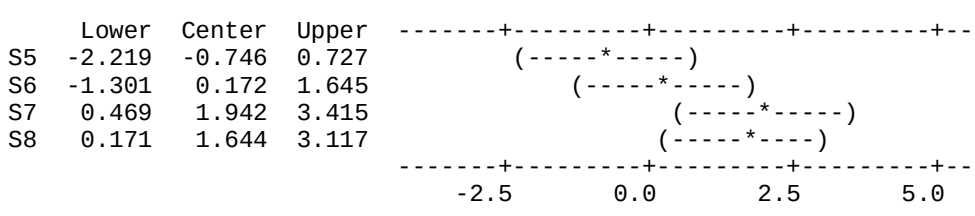
One-way ANOVA: fiber diameter

```
Source   DF       SS     MS     F      P
Factor    7   216.75  30.96  8.92  0.000
Error   232   805.50   3.47
Total   239  1022.25

S = 1.863   R-Sq = 21.20%   R-Sq(adj) = 18.83%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N   Mean  StDev  ---------+---------+---------+---------+
S1     30  3.149  0.721  (----*-----)
S2     30  4.854  1.635               (----*-----)
S3     30  4.067  1.153         (-----*----)
S4     30  3.950  1.561        (-----*-----)
S5     30  3.204  0.902  (-----*----)
S6     30  4.122  1.335          (----*-----)
S7     30  5.892  1.879                      (----*-----)
S8     30  5.594  3.833                    (-----*----)
                          ---------+---------+---------+---------+
                                 3.6       4.8       6.0       7.2

Pooled StDev = 1.863


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.75%


S1 subtracted from:

     Lower  Center  Upper  -------+---------+---------+---------+--
S2   0.232   1.705  3.178                   (-----*-----)
S3  -0.555   0.918  2.391               (-----*-----)
S4  -0.672   0.801  2.274              (-----*-----)
S5  -1.418   0.055  1.528          (-----*-----)
S6  -0.501   0.972  2.445               (-----*-----)
S7   1.270   2.743  4.216                     (-----*-----)
S8   0.971   2.444  3.917                   (-----*-----)
                          -------+---------+---------+---------+--
                              -2.5       0.0       2.5       5.0


S2 subtracted from:

     Lower  Center   Upper  -------+---------+---------+---------+--
S3  -2.260  -0.787   0.686         (-----*-----)
S4  -2.377  -0.904   0.569        (-----*-----)
S5  -3.123  -1.650  -0.177      (----*-----)
S6  -2.206  -0.733   0.740         (-----*-----)
S7  -0.435   1.038   2.511               (-----*-----)
S8  -0.734   0.739   2.212              (-----*-----)
                          -------+---------+---------+---------+--
                              -2.5       0.0       2.5       5.0
```

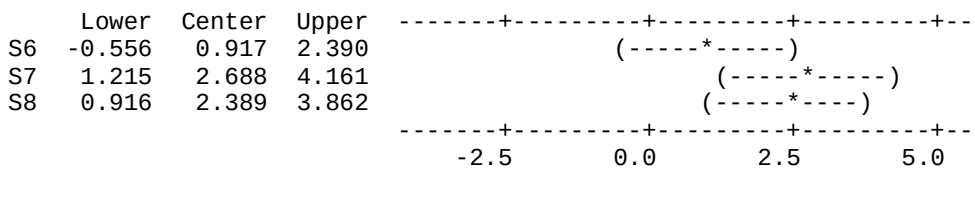
S3 subtracted from:



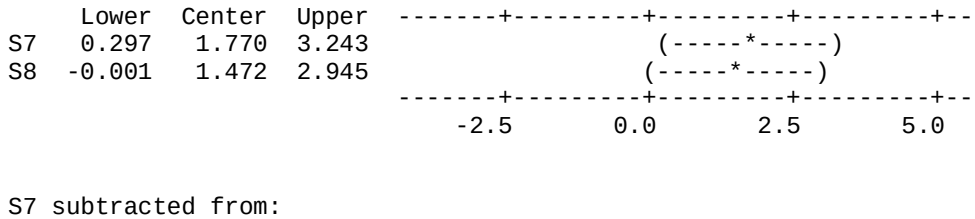
S4 subtracted from:



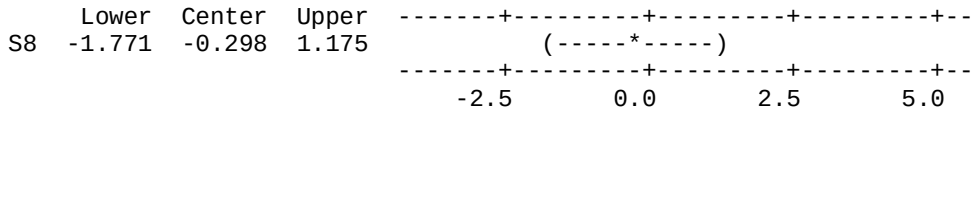
S5 subtracted from:



S6 subtracted from:



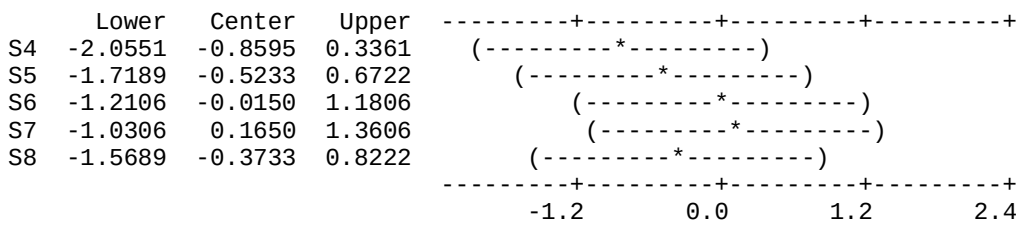
S7 subtracted from:



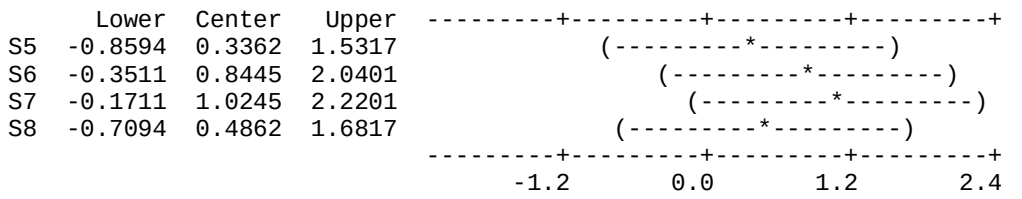
One-way ANOVA: Stress

```
Source  DF      SS     MS     F      P
Factor   7   5.901  0.843  2.01  0.078
Error   40  16.791  0.420
Total   47  22.692

S = 0.6479   R-Sq = 26.01%   R-Sq(adj) = 13.06%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean   StDev   -+---------+---------+---------+--------
S1     6  1.1363  0.3192    (--------*--------)
S2     6  1.6463  1.0160             (-------*--------)
S3     6  1.9223  1.1277                 (--------*--------)
S4     6  1.0628  0.3230   (--------*--------)
S5     6  1.3990  0.5784        (--------*--------)
S6     6  1.9073  0.5189                 (--------*--------)
S7     6  2.0873  0.3356                    (--------*--------)
S8     6  1.5490  0.3629           (--------*--------)
                          -+---------+---------+---------+--------
                         0.60      1.20      1.80      2.40

Pooled StDev = 0.6479


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.73%


S1 subtracted from:

      Lower   Center   Upper  ---------+---------+---------+---------+
S2  -0.6856   0.5100  1.7056               (---------*---------)
S3  -0.4096   0.7860  1.9816                  (---------*---------)
S4  -1.2691  -0.0735  1.1221          (---------*---------)
S5  -0.9329   0.2627  1.4582             (---------*---------)
S6  -0.4246   0.7710  1.9666                 (---------*---------)
S7  -0.2446   0.9510  2.1466                   (---------*---------)
S8  -0.7829   0.4127  1.6082              (---------*---------)
                           ---------+---------+---------+---------+
                                 -1.2       0.0       1.2       2.4


S2 subtracted from:

      Lower   Center   Upper  ---------+---------+---------+---------+
S3  -0.9196   0.2760  1.4716             (---------*---------)
S4  -1.7791  -0.5835  0.6121      (---------*---------)
S5  -1.4429  -0.2473  0.9482         (---------*---------)
S6  -0.9346   0.2610  1.4566             (---------*---------)
S7  -0.7546   0.4410  1.6366               (---------*---------)
S8  -1.2929  -0.0973  1.0982          (---------*---------)
                           ---------+---------+---------+---------+
                                 -1.2       0.0       1.2       2.4
```

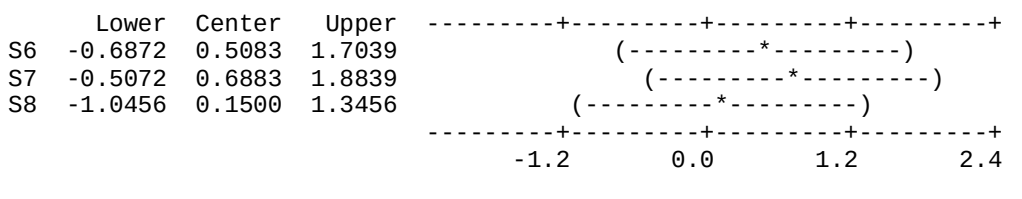
S3 subtracted from:



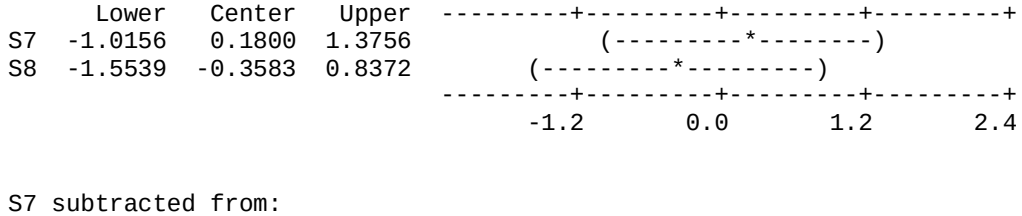
S4 subtracted from:



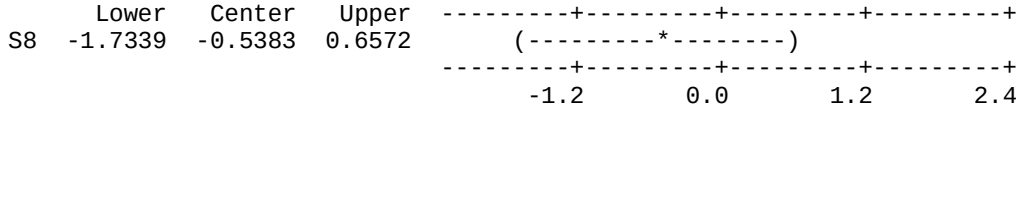
S5 subtracted from:



S6 subtracted from:



S7 subtracted from:



One-way ANOVA: Strain

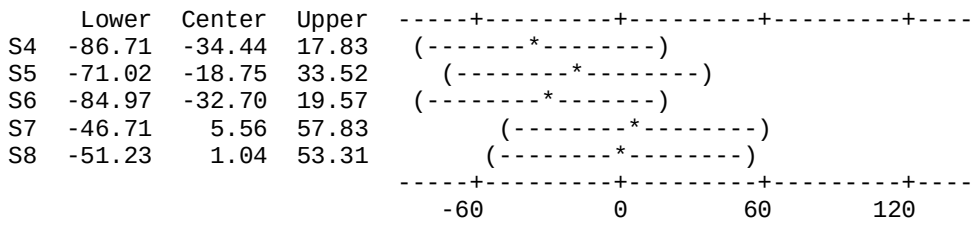
```
Source  DF        SS        MS     F      P
Factor   7  0.003182  0.000455  2.04  0.073
Error   40  0.008901  0.000223
Total   47  0.012083

S = 0.01492   R-Sq = 26.34%   R-Sq(adj) = 13.44%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev  --------+---------+---------+---------+-
S1     6  0.02833  0.00665          (-------*-------)
S2     6  0.03967  0.03828                  (-------*--------)
S3     6  0.02250  0.00723       (-------*-------)
S4     6  0.01767  0.00745    (-------*-------)
S5     6  0.02250  0.00509       (-------*-------)
S6     6  0.03667  0.00845               (-------*--------)
S7     6  0.02750  0.00606         (-------*--------)
S8     6  0.01467  0.00539  (-------*-------)
                          --------+---------+---------+---------+-
                                0.015     0.030     0.045     0.060

Pooled StDev = 0.01492


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.73%


S1 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S2  -0.01619   0.01133  0.03886              (--------*--------)
S3  -0.03336  -0.00583  0.02169         (--------*--------)
S4  -0.03819  -0.01067  0.01686       (--------*---------)
S5  -0.03336  -0.00583  0.02169         (--------*--------)
S6  -0.01919   0.00833  0.03586              (--------*--------)
S7  -0.02836  -0.00083  0.02669           (--------*--------)
S8  -0.04119  -0.01367  0.01386      (--------*---------)
                                   --------+---------+---------+---------+-
                                        -0.030     0.000     0.030     0.060


S2 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S3  -0.04469  -0.01717  0.01036     (--------*--------)
S4  -0.04953  -0.02200  0.00553   (---------*--------)
S5  -0.04469  -0.01717  0.01036     (--------*--------)
S6  -0.03053  -0.00300  0.02453          (--------*--------)
S7  -0.03969  -0.01217  0.01536       (--------*--------)
S8  -0.05253  -0.02500  0.00253  (---------*--------)
                                   --------+---------+---------+---------+-
                                        -0.030     0.000     0.030     0.060
```

```
S3 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S4  -0.03236  -0.00483  0.02269         (--------*---------)
S5  -0.02753   0.00000  0.02753           (--------*--------)
S6  -0.01336   0.01417  0.04169                (--------*--------)
S7  -0.02253   0.00500  0.03253             (---------*--------)
S8  -0.03536  -0.00783  0.01969        (--------*---------)
                                   --------+---------+---------+---------+-
                                        -0.030     0.000     0.030     0.060


S4 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S5  -0.02269   0.00483  0.03236            (---------*--------)
S6  -0.00853   0.01900  0.04653                 (--------*---------)
S7  -0.01769   0.00983  0.03736              (--------*--------)
S8  -0.03053  -0.00300  0.02453          (--------*--------)
                                   --------+---------+---------+---------+-
                                        -0.030     0.000     0.030     0.060


S5 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S6  -0.01336   0.01417  0.04169                (--------*--------)
S7  -0.02253   0.00500  0.03253            (---------*--------)
S8  -0.03536  -0.00783  0.01969        (--------*---------)
                                   --------+---------+---------+---------+-
                                        -0.030     0.000     0.030     0.060


S6 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S7  -0.03669  -0.00917  0.01836        (--------*--------)
S8  -0.04953  -0.02200  0.00553   (---------*--------)
                                   --------+---------+---------+---------+-
                                        -0.030     0.000     0.030     0.060


S7 subtracted from:

       Lower    Center    Upper  --------+---------+---------+---------+-
S8  -0.04036  -0.01283  0.01469      (--------*--------)
                                   --------+---------+---------+---------+-
                                        -0.030     0.000     0.030     0.060
```

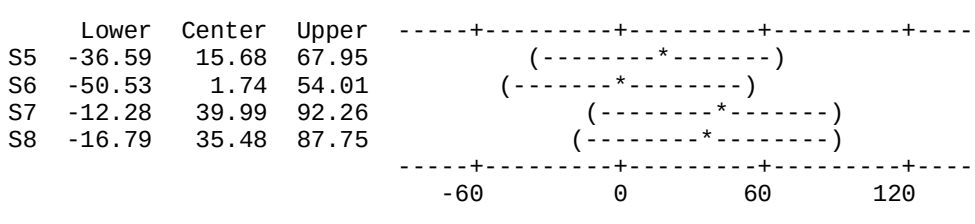
One-way ANOVA: Modulus

```
Source  DF     SS    MS     F      P
Factor   7  18245  2606  3.25  0.008
Error   40  32095   802
Total   47  50340

S = 28.33   R-Sq = 36.24%   R-Sq(adj) = 25.09%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  --+---------+---------+---------+-------
S1     6   47.13   6.46  (-------*-------)
S2     6   93.64  46.67                (-------*-------)
S3     6   99.28  45.64                  (-------*-------)
S4     6   64.84  15.30        (-------*------)
S5     6   80.52  20.33             (-------*-------)
S6     6   66.58  18.81        (-------*-------)
S7     6  104.84  18.74                   (-------*-------)
S8     6  100.32  27.64                  (------*-------)
                        --+---------+---------+---------+-------
                         30        60        90       120

Pooled StDev = 28.33


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.73%


S1 subtracted from:

     Lower  Center   Upper  -----+---------+---------+---------+----
S2   -5.77   46.50   98.77               (--------*-------)
S3   -0.13   52.14  104.41                (--------*-------)
S4  -34.56   17.71   69.98          (--------*--------)
S5  -18.88   33.39   85.66             (--------*-------)
S6  -32.82   19.45   71.72           (-------*--------)
S7    5.43   57.70  109.97                 (--------*-------)
S8    0.92   53.19  105.46                (--------*--------)
                          -----+---------+---------+---------+----
                             -60         0        60       120


S2 subtracted from:

     Lower  Center  Upper  -----+---------+---------+---------+----
S3  -46.63    5.64  57.91        (--------*--------)
S4  -81.07  -28.80  23.47  (--------*--------)
S5  -65.38  -13.11  39.16     (--------*--------)
S6  -79.33  -27.06  25.21   (-------*--------)
S7  -41.07   11.20  63.47         (--------*--------)
S8  -45.59    6.68  58.95        (--------*--------)
                          -----+---------+---------+---------+----
                             -60         0        60       120
```

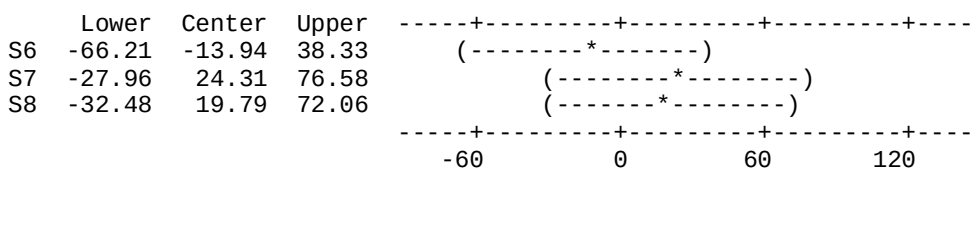
S3 subtracted from:



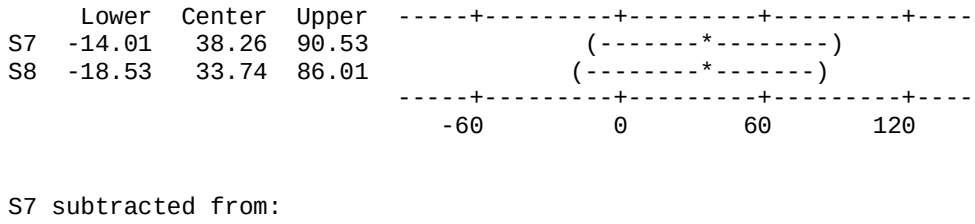
S4 subtracted from:



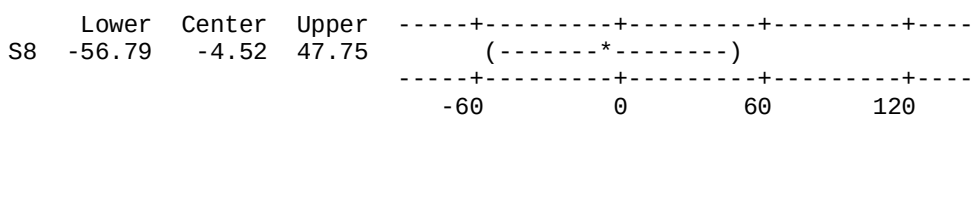
S5 subtracted from:



S6 subtracted from:

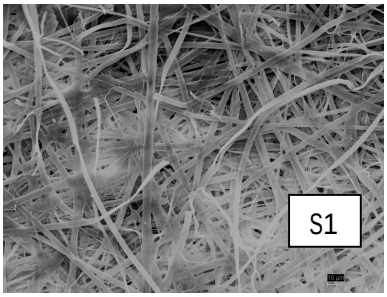


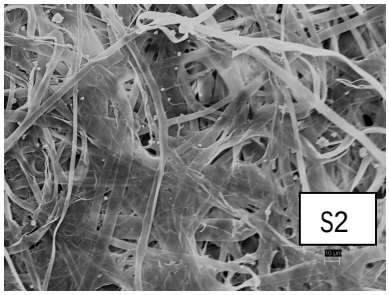
S7 subtracted from:

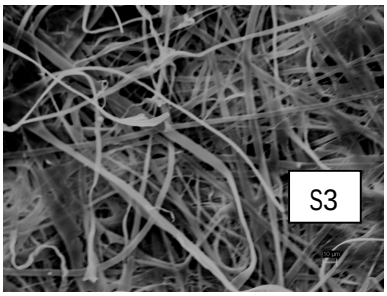


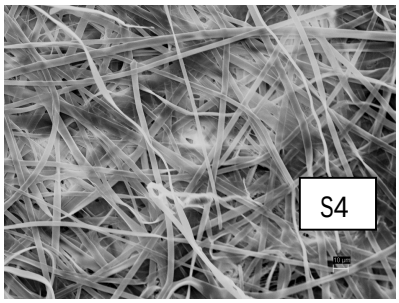
Appendix C (Data for the scaffolds crosslinked by the vapor method)

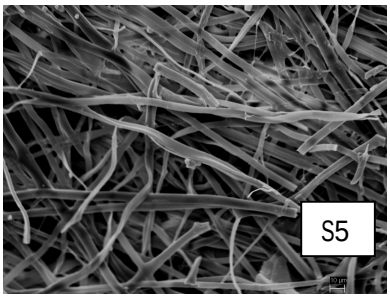
SEM images (The scale bar in all the images is 10 μm)

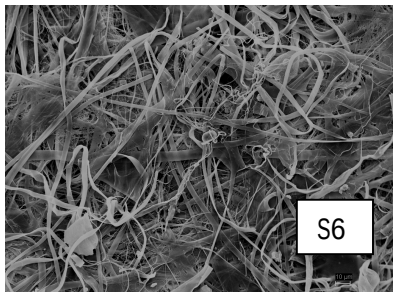


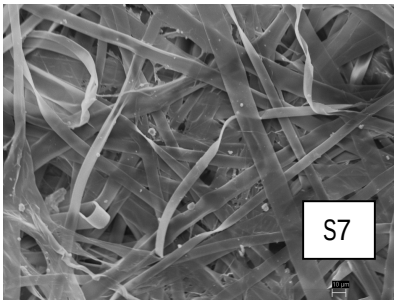


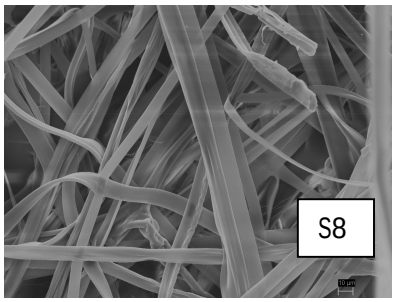




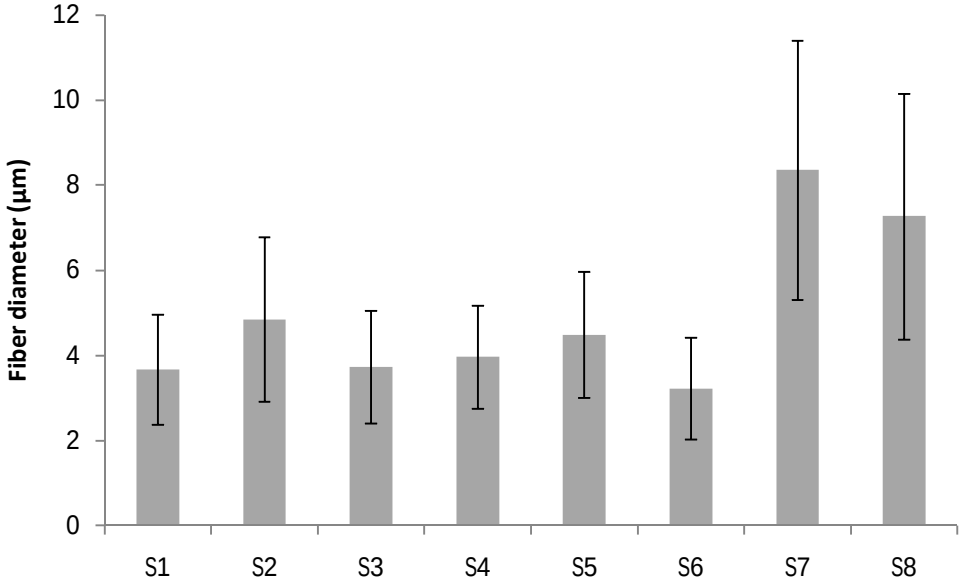




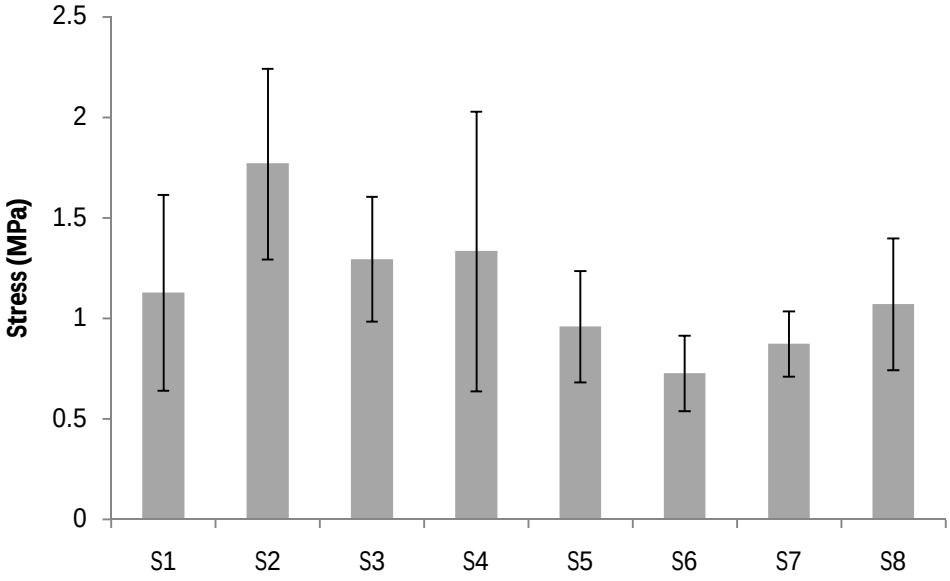




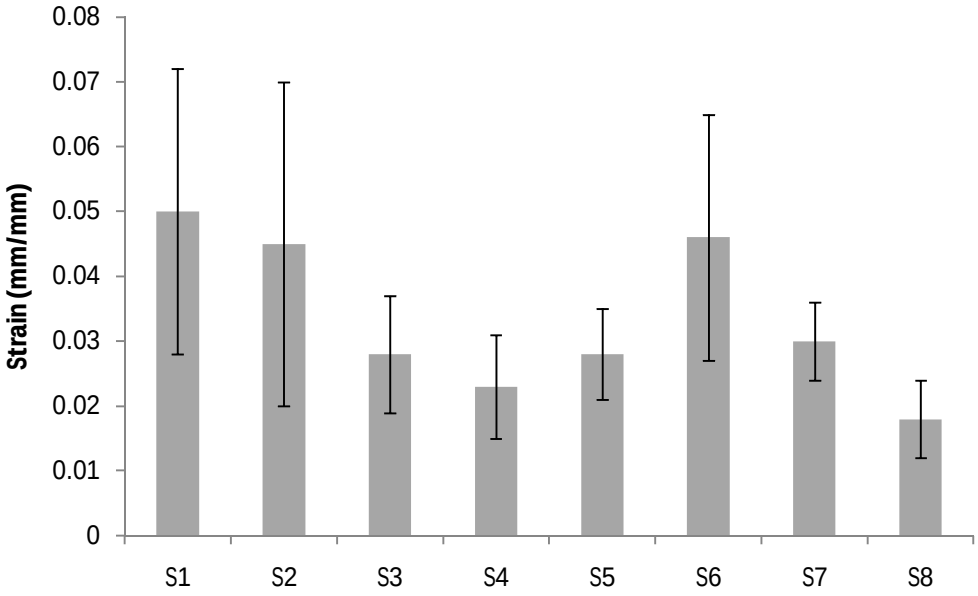
Fiber diameter



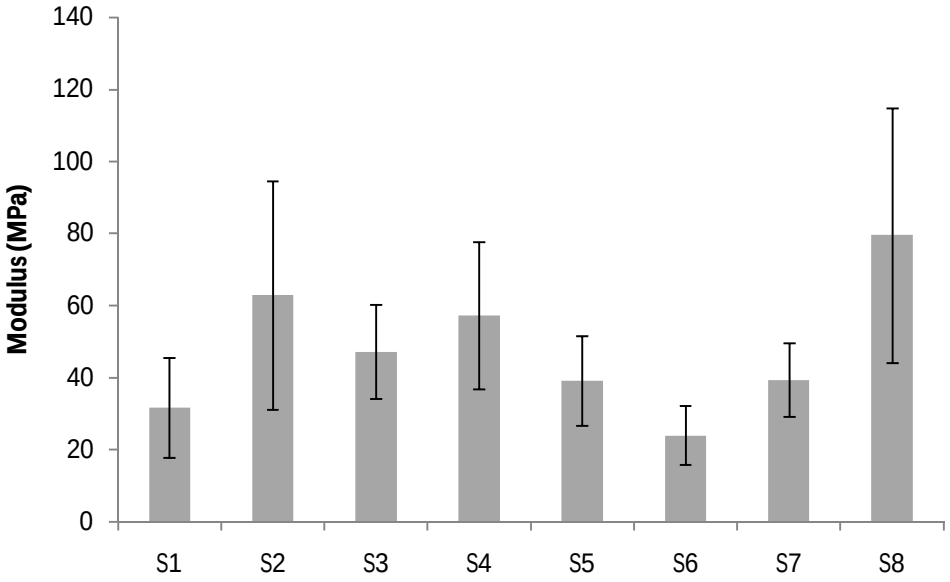
Stress



Strain



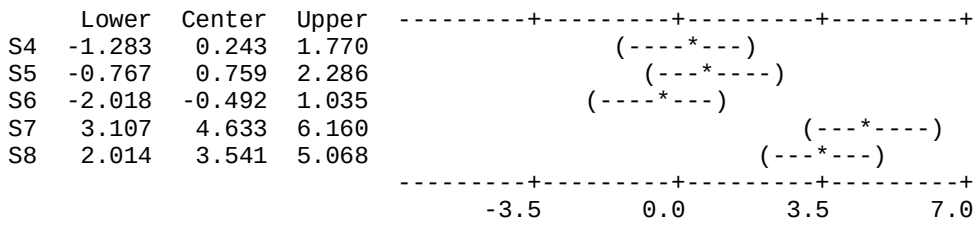
Modulus



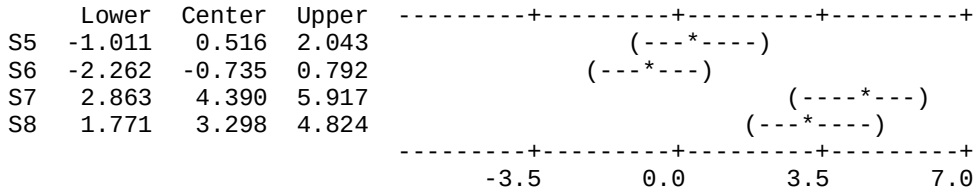
One-way ANOVA: fiber diameter

```
Source   DF       SS      MS      F      P
Factor    7   728.15  104.02  27.89  0.000
Error   232   865.31    3.73
Total   239  1593.46

S = 1.931   R-Sq = 45.70%   R-Sq(adj) = 44.06%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level   N   Mean  StDev  -------+---------+---------+---------+--
S1     30  3.666  1.287    (--*---)
S2     30  4.845  1.928          (--*---)
S3     30  3.727  1.318    (---*--)
S4     30  3.970  1.214     (---*--)
S5     30  4.486  1.481        (--*---)
S6     30  3.235  1.186  (--*---)
S7     30  8.360  3.047                          (---*--)
S8     30  7.268  2.894                      (--*---)
                         -------+---------+---------+---------+--
                              4.0       6.0       8.0      10.0

Pooled StDev = 1.931


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.75%


S1 subtracted from:

     Lower  Center  Upper  ---------+---------+---------+---------+
S2  -0.348   1.179  2.706                   (---*----)
S3  -1.466   0.060  1.587                (---*----)
S4  -1.223   0.304  1.830                 (---*---)
S5  -0.707   0.820  2.346                  (---*----)
S6  -1.958  -0.431  1.095              (----*---)
S7   3.167   4.694  6.220                              (---*----)
S8   2.075   3.601  5.128                           (---*----)
                          ---------+---------+---------+---------+
                                -3.5       0.0       3.5       7.0


S2 subtracted from:

     Lower  Center   Upper  ---------+---------+---------+---------+
S3  -2.645  -1.119   0.408            (----*---)
S4  -2.402  -0.875   0.651             (---*----)
S5  -1.886  -0.359   1.167               (---*---)
S6  -3.137  -1.610  -0.084           (---*----)
S7   1.988   3.515   5.041                           (---*---)
S8   0.896   2.422   3.949                        (---*---)
                           ---------+---------+---------+---------+
                                 -3.5       0.0       3.5       7.0
```

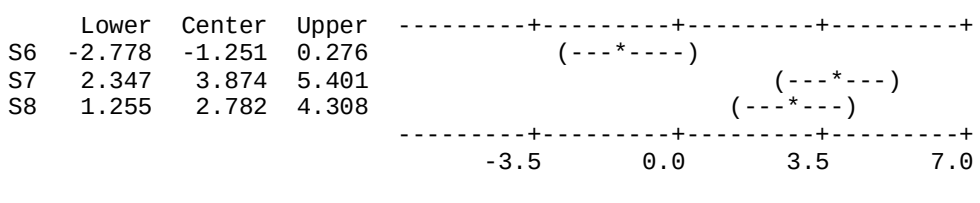
S3 subtracted from:



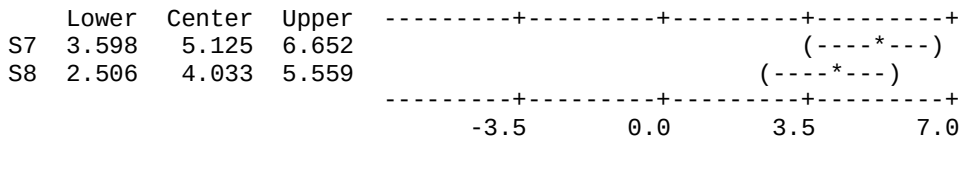
S4 subtracted from:



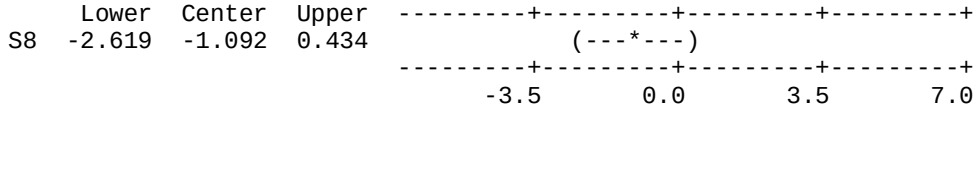
S5 subtracted from:



S6 subtracted from:



S7 subtracted from:



One-way ANOVA: Stress

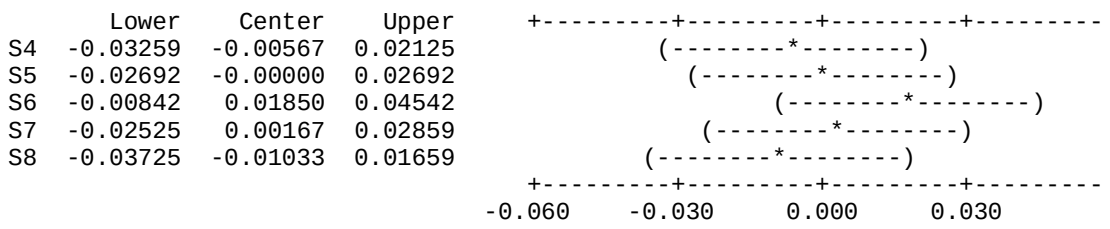
```
Source  DF      SS     MS     F      P
Factor   7   4.406  0.629  3.91  0.002
Error   40   6.437  0.161
Total   47  10.843

S = 0.4011   R-Sq = 40.64%   R-Sq(adj) = 30.25%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean   StDev  --+---------+---------+---------+-------
S1     6  1.1302  0.4865         (------*-----)
S2     6  1.7692  0.4763                      (-----*------)
S3     6  1.2947  0.3091             (------*------)
S4     6  1.3357  0.6963              (------*-----)
S5     6  0.9598  0.2757       (-----*------)
S6     6  0.7283  0.1873  (------*-----)
S7     6  0.8755  0.1609     (------*-----)
S8     6  1.0722  0.3263         (-----*------)
                         --+---------+---------+---------+-------
                         0.50      1.00      1.50      2.00

Pooled StDev = 0.4011


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.73%


S1 subtracted from:

      Lower   Center   Upper  --------+---------+---------+---------+-
S2  -0.1012   0.6390  1.3792                   (------*-------)
S3  -0.5757   0.1645  0.9047              (-------*------)
S4  -0.5347   0.2055  0.9457               (------*------)
S5  -0.9106  -0.1703  0.5699           (------*-------)
S6  -1.1421  -0.4018  0.3384         (------*------)
S7  -0.9949  -0.2547  0.4856          (------*-------)
S8  -0.7982  -0.0580  0.6822            (------*-------)
                           --------+---------+---------+---------+-
                                -1.0       0.0       1.0       2.0


S2 subtracted from:

      Lower   Center    Upper  --------+---------+---------+---------+-
S3  -1.2147  -0.4745   0.2657        (-------*-------)
S4  -1.1737  -0.4335   0.3067        (-------*------)
S5  -1.5496  -0.8093  -0.0691     (------*------)
S6  -1.7811  -1.0408  -0.3006  (-------*------)
S7  -1.6339  -0.8937  -0.1534    (------*------)
S8  -1.4372  -0.6970   0.0432      (------*------)
                           --------+---------+---------+---------+-
                                -1.0       0.0       1.0       2.0
```

```
S3 subtracted from:

      Lower   Center   Upper  --------+---------+---------+---------+-
S4  -0.6992   0.0410  0.7812            (------*-------)
S5  -1.0751  -0.3348  0.4054          (-------*------)
S6  -1.3066  -0.5663  0.1739       (------*-------)
S7  -1.1594  -0.4192  0.3211         (-------*------)
S8  -0.9627  -0.2225  0.5177           (-------*------)
                              --------+---------+---------+---------+-
                                   -1.0       0.0       1.0       2.0


S4 subtracted from:

      Lower   Center   Upper  --------+---------+---------+---------+-
S5  -1.1161  -0.3758  0.3644          (------*-------)
S6  -1.3476  -0.6073  0.1329       (------*------)
S7  -1.2004  -0.4602  0.2801         (------*-------)
S8  -1.0037  -0.2635  0.4767           (------*-------)
                              --------+---------+---------+---------+-
                                   -1.0       0.0       1.0       2.0


S5 subtracted from:

      Lower   Center   Upper  --------+---------+---------+---------+-
S6  -0.9717  -0.2315  0.5087          (-------*------)
S7  -0.8246  -0.0843  0.6559            (------*-------)
S8  -0.6279   0.1123  0.8526              (------*-------)
                              --------+---------+---------+---------+-
                                   -1.0       0.0       1.0       2.0


S6 subtracted from:

      Lower  Center   Upper  --------+---------+---------+---------+-
S7  -0.5931  0.1472  0.8874             (------*-------)
S8  -0.3964  0.3438  1.0841               (------*-------)
                              --------+---------+---------+---------+-
                                   -1.0       0.0       1.0       2.0


S7 subtracted from:

      Lower  Center   Upper  --------+---------+---------+---------+-
S8  -0.5436  0.1967  0.9369             (------*------)
                              --------+---------+---------+---------+-
                                   -1.0       0.0       1.0       2.0
```

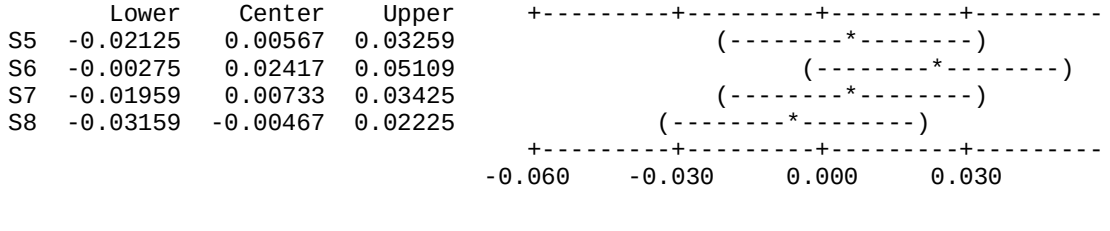
One-way ANOVA: Strain

```
Source  DF        SS        MS     F      P
Factor   7  0.006165  0.000881  4.14  0.002
Error   40  0.008512  0.000213
Total   47  0.014677

S = 0.01459   R-Sq = 42.01%   R-Sq(adj) = 31.86%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ------+---------+---------+---------+---
S1     6  0.05033  0.02170                       (-------*-------)
S2     6  0.04467  0.02491                   (-------*-------)
S3     6  0.02800  0.00876         (-------*-------)
S4     6  0.02233  0.00769     (-------*-------)
S5     6  0.02800  0.00699         (-------*-------)
S6     6  0.04650  0.01875                    (-------*-------)
S7     6  0.02967  0.00592          (-------*-------)
S8     6  0.01767  0.00631  (-------*-------)
                           ------+---------+---------+---------+---
                               0.015     0.030     0.045     0.060

Pooled StDev = 0.01459


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.73%


S1 subtracted from:

       Lower    Center     Upper     +---------+---------+---------+---------
S2  -0.03259  -0.00567   0.02125               (--------*--------)
S3  -0.04925  -0.02233   0.00459          (--------*--------)
S4  -0.05492  -0.02800  -0.00108        (--------*--------)
S5  -0.04925  -0.02233   0.00459          (--------*--------)
S6  -0.03075  -0.00383   0.02309                (--------*--------)
S7  -0.04759  -0.02067   0.00625          (--------*--------)
S8  -0.05959  -0.03267  -0.00575     (--------*--------)
                                    +---------+---------+---------+---------
                                 -0.060    -0.030     0.000     0.030


S2 subtracted from:

       Lower    Center     Upper     +---------+---------+---------+---------
S3  -0.04359  -0.01667   0.01025           (--------*--------)
S4  -0.04925  -0.02233   0.00459          (--------*--------)
S5  -0.04359  -0.01667   0.01025           (--------*--------)
S6  -0.02509   0.00183   0.02875                 (--------*--------)
S7  -0.04192  -0.01500   0.01192            (--------*--------)
S8  -0.05392  -0.02700  -0.00008        (--------*--------)
                                    +---------+---------+---------+---------
                                 -0.060    -0.030     0.000     0.030
```

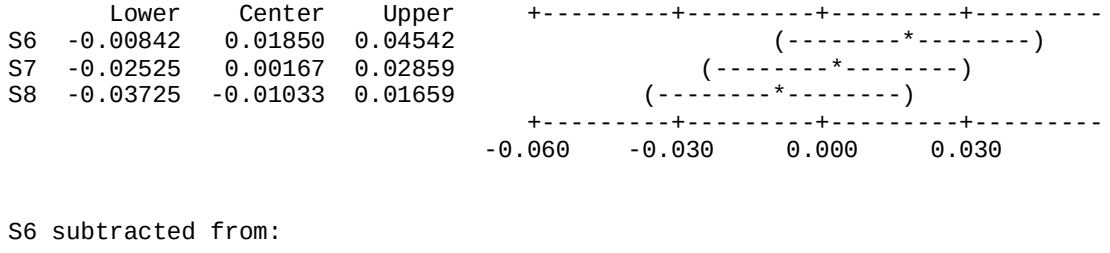
S3 subtracted from:



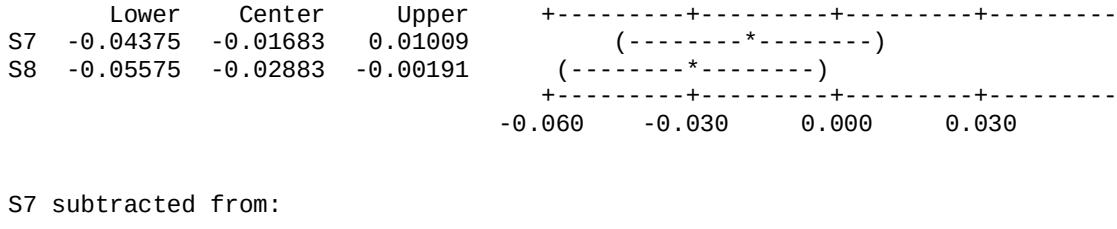
S4 subtracted from:



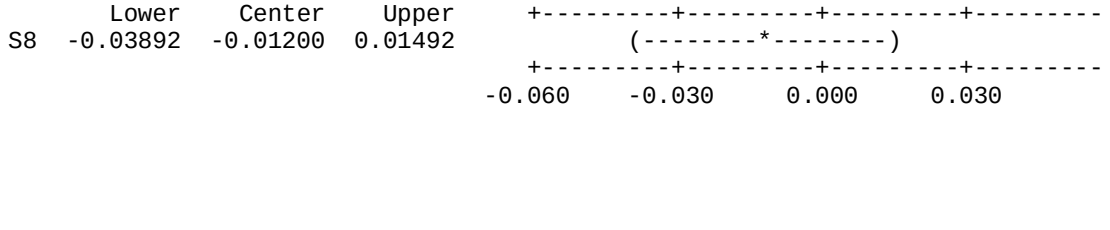
S5 subtracted from:



S6 subtracted from:



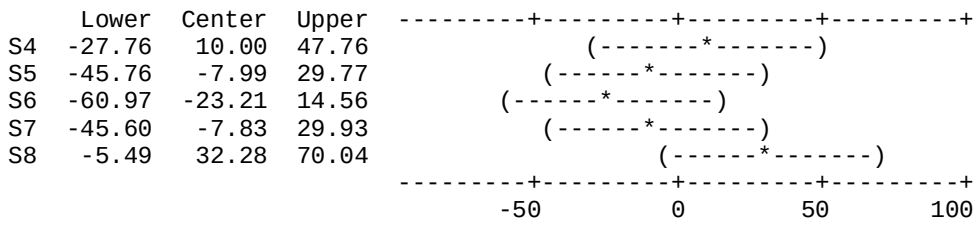
S7 subtracted from:



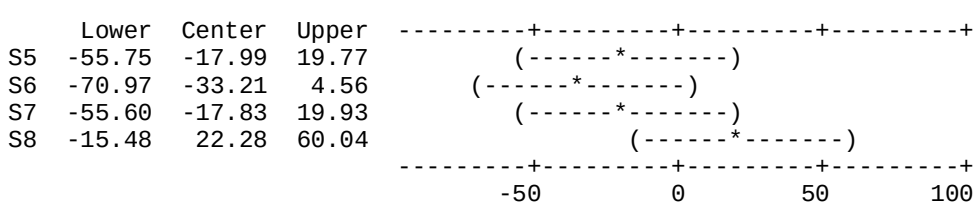
One-way ANOVA: modulus

```
Source  DF     SS    MS     F      P
Factor   7  13749  1964  4.69  0.001
Error   40  16753   419
Total   47  30502

S = 20.47   R-Sq = 45.08%   R-Sq(adj) = 35.47%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N   Mean  StDev  -------+---------+---------+---------+--
S1     6  31.73  13.89     (------*-----)
S2     6  62.95  31.61                (------*------)
S3     6  47.30  13.03           (------*------)
S4     6  57.30  20.36                (------*------)
S5     6  39.31  12.39        (------*-----)
S6     6  24.10   8.31  (------*-----)
S7     6  39.47  10.17        (------*------)
S8     6  79.58  35.32                        (------*------)
                        -------+---------+---------+---------+--
                               25        50        75       100

Pooled StDev = 20.47


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99.73%


S1 subtracted from:

     Lower  Center  Upper  ---------+---------+---------+---------+
S2   -6.55   31.21  68.98                    (-------*-------)
S3  -22.19   15.57  53.34                (-------*-------)
S4  -12.19   25.57  63.34                  (-------*-------)
S5  -30.18    7.58  45.35              (-------*------)
S6  -45.40   -7.64  30.13           (------*-------)
S7  -30.03    7.74  45.50              (-------*------)
S8   10.09   47.85  85.61                       (-------*------)
                          ---------+---------+---------+---------+
                                 -50         0        50       100


S2 subtracted from:

     Lower  Center  Upper  ---------+---------+---------+---------+
S3  -53.41  -15.64  22.12          (-------*------)
S4  -43.41   -5.64  32.12            (-------*------)
S5  -61.40  -23.63  14.13         (------*-------)
S6  -76.61  -38.85  -1.09      (------*-------)
S7  -61.24  -23.48  14.29         (------*-------)
S8  -21.13   16.64  54.40                 (------*-------)
                          ---------+---------+---------+---------+
                                 -50         0        50       100
```

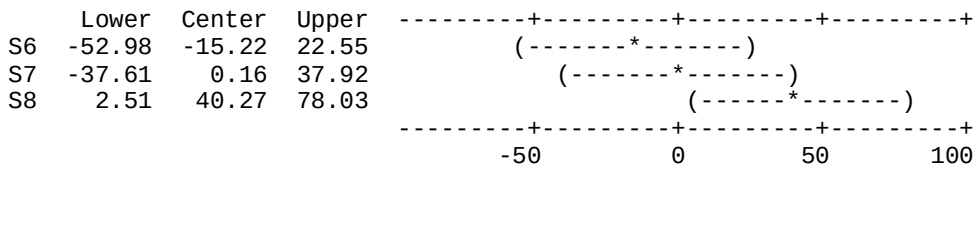
S3 subtracted from:



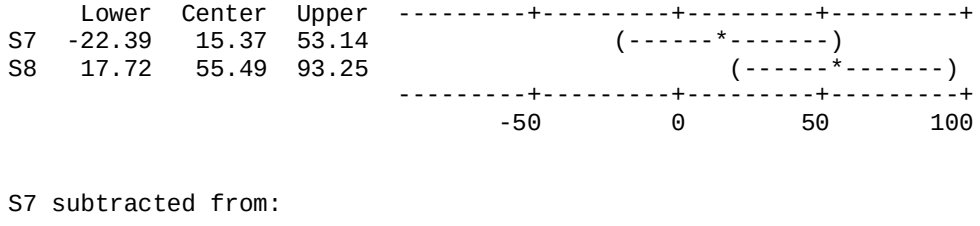
S4 subtracted from:



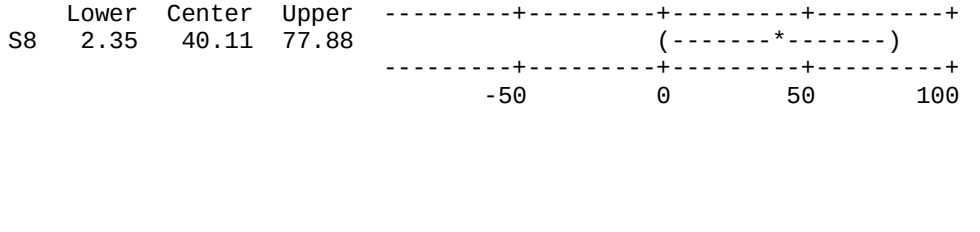
S5 subtracted from:



S6 subtracted from:



S7 subtracted from:



VITA

Alpana A. Dongargaonkar was born on October 24, 1985 in Mumbai, India. She graduated from Menon High School, Mumbai in 2003. She received her Bachelor of Technology in Biotechnology from ICFAI Institute of Science and Technology, Hyderabad, India in 2007. She began her graduate studies in Biomedical Engineering at Virginia Commonwealth University in Fall 2008. During her time at VCU, her research was focused on tissue engineering and drug delivery. She is very keen on expanding her knowledge in the field of stem cell and tissue engineering.